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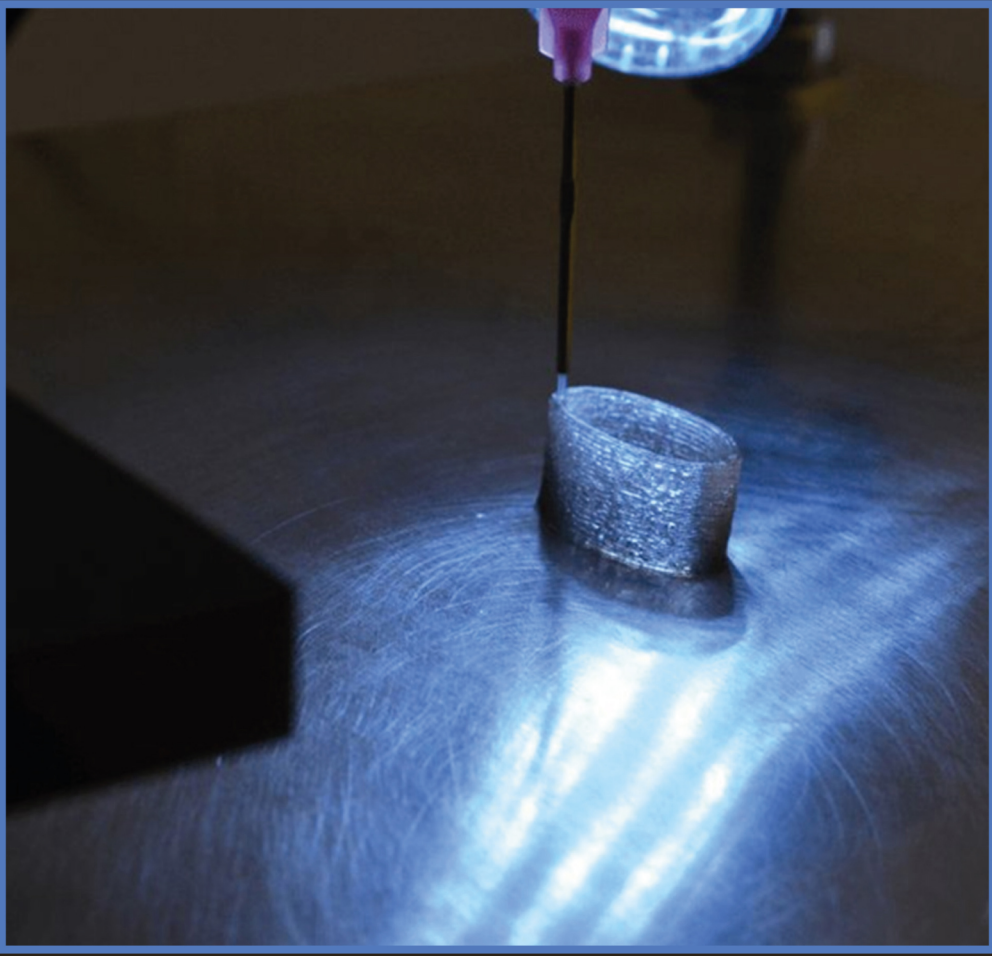

3D PRINTING IN MEDICINE AND SURGERY

APPLICATIONS IN HEALTHCARE

Edited by
DANIEL J. THOMAS
DEEPTI SINGH

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3D Printing in Medicine and Surgery

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Woodhead Publishing Series in Biomaterials

3D Printing in Medicine and Surgery

Applications in Healthcare

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About the editors

Dr. Daniel J. Thomas BEng (Hons) MSc EngD CEnv CSci CEng FBCS, is an interdisciplinary researcher and writer who is from an academic background in 3D Printing, Engineering, and Computational Design. This book "3D Printing in Medicine and Surgery" focuses on his current research as well as the research of numerous researchers who are engaged in developing new 3D printing technologies that are currently treating patients and will lead toward the development of future treatments.

Dr. Deepti Singh MSc PhD, is a bioengineer who has been exploring application of polymeric materials in restoring or repairing injured tissues and organs. With PhD in genetics and biotechnology, she has worked extensively for last decade to develop functional biomimetic that can enhance differentiation of embryonic stem cells into the desired linage. This book focuses on interesting and new aspects of biomaterial designing and its applications. With emergence of bio-ink, 3D printing could potentially change the entire approach of regenerative medicine in which instead of restoring and repairing, the disease tissue can be replaced.

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Preface

This book 3D Printing in Medicine and Surgery focuses on the area of 3D printing in medicine, specifically for surgical and enhanced-medical applications. It is written in the form of a textbook with a practical approach toward the use of 3D printing as part of future medical procedures. This focuses on techniques and current uses of 3D printing technology, which advance medical science and the innovations, which impact medicine. This is in the form of detailing state-of-the-art approaches, which are specifically used as part of the body's structural and biomechanical systems.

3D printing is a critical future tool for the enhancing medical treatment. It is a paradigm that will be important toward translational treatment and in supporting the development of future medicine. The underlying theme of this book is processes including; Techniques for 3D Printing for Augmenting Medicine, Applications of 3D Printing in Transplantation Procedures, Future 3D Printing Technology and Regulation.

This book also focuses on semitraditional approaches to 3D printing, as well as 3D approaches and practical methods. Toward the end of this book a number of case studies are authored on the uses of 3D printing to generate a range of constructs used for transplantation and modeling.

The revolutionary arena of 3D printing is one, which is accelerating fast, and one which promises to yield future treatments for patients. 3D Printing in Medicine and Surgery features practical guidance, processes for preparing 3D printable materials, together with a foundation of background knowledge in the area of 3D Printing.

This book is as a result a useful handbook, which presents medical practitioners to a range of practical methods. It will contain applied knowledge including how 3D printing equipment can be used within the hospital environment. It equips readers with a toolbox of different methods for 3D printing medical structures. This covers a range of polymers, alloys, biological scaffolds, biomaterials, and methods used by current medical practitioners.

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Introduction

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Chapter outline

1.1 Introduction

The interdisciplinary research area of 3D printing in medicine and surgery has developed solidly and rapidly over the past 20 years. The paradigm of personalized medicine is now starting to come to fruition through the use of new 3D additive manufacturing technologies. This offers a shift in a new revolution in precision medicine that have become easier to realize and implement. 3D printing intersects the various medical disciplines to enable a patient-centric approach toward treatment. There is currently an increased research effort focused on the application of 3D printing research to a range of healthcare applications.

It is through this use of new technologies such as 3D printing that personalized medicine can now be delivered. This is through the dreams of clinicians, the concepts of imaginative researchers and into the hands of pioneer medics. In this book, we will discuss the various technologies that make up 3D printing in medicine and surgery. We will consider the use of ground-breaking technologies and techniques that will help treat numerous patients both now and well into the future.

3D printing is evolving from convention preoperative and interoperative models, through to the production of custom-made implants and regenerative tissue systems. Staring with patient centric models, these are used to create complex geometries that mimic the structure of the human body. The field of 3D printing in medicine and surgery brings together specialists in the field of science, medicine, the various engineering disciplines and physics. Working together, this offers a rapid means to capitalize on the developing of 3D printing in the traditional engineering-related disciplines.

Excitingly, 3D printing in medicine is not just an out of the box solution. It requires ingenuity, inventiveness and a pioneering spirit that could change the world of healthcare. Throughout this book, we will discuss the various 3D printing technologies that specialists are developing every day. Together with novel and advanced materials that are used as printable substrates. The synergy between materials, technology and healthcare provides a limitless opportunity toward the development of new treatments.

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Creating the technologies that help bridge the gap between science fiction and reality helps ensure that the healthcare sector will see a distinct change in the future. A future where medical treatment become patient specific and not a traditional one size fits all solution. This poses key advantages toward providing long-term treatment. Such a vision requires applications that will require high performance materials that can be 3D printed. The paramount technology is that of 3D-based bioprinting solutions that have the definitive potential to help enhance and sustain lives. Although this technology is still in its infancy, it is an exciting future prospect.

Although 3D printing is collectively classed as an additive process that precisely builds complex objects and geometries one layer at a time. The exciting prospect is when this concept is placed into the hands of creative and daring people who push it toward the next frontier. Although polymers remain the key 3D printable material, it is when more complex materials are added to the equation that exciting and adaptive outcomes are developed. Throughout this book, we will demonstrate this through the experiments and successes of others as well as ourselves.

3D printing also provides the benefit of affordable healthcare, in which the customization of components can be done relatively easily. Implants and components can be made quickly that are user developed. Through the use of advanced polymers, components can be manufactured that are both lighter and stronger. And 3D printed parts can be made to have internal integrated moving parts and these can all be manufactured at the point of care.

We welcome you to this book 3D printing in medicine and surgery. We hope that you will be as enthusiastic about this technology as we are. In this hope, we hope that you will go on to explore 3D printing in medicine and translate it to your own requirements that go on to help patients and those that are in need of this technology.

Your spirit to clinical development will no doubt make great strides in the improvement of healthcare treatments.

Thanks for reading and wishing you an exciting journey of discovery.

Clinical uses of 3D printing

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Chapter outline

2.1 Introduction

With the growing push of the application of effective technology, 3D printing technology has recently been applied and utilized in different fields including industry, education, and medicine. It includes a number of manufacturing technologies that are able to produce three-dimensional (3D) physical models through handling data in its digital form [1]. 3DP ability's to deliver personalized, patient-centered medicine based on patient's anatomy scan data have been the driving force behind its widespread uses in different disciplines such as neurosurgery, cardiovascular, maxillofacial, transplant and general surgery, and orthopedics [2–5]. A recent search of Pubmed using combination of the Mesh terms "3D printing; 3dp; medicine; medical specialties," has revealed increasing number of case reports and clinical series publications over the last two decades, ranging from 21 in 2002, to 88 in 2014 and reached more than 1220 publications in 2018 covering most of medical disciplines. A number of remarkable societies have embraced the idea of using this new technology [5]. 3D printing common clinical uses among medical specialties included virtual pre-surgical assessment and planning; inter-surgery guidance and customized medical implants; patient consenting; and medical education and training. Current chapter will provide illustrative examples of the clinical uses.

2.2 Pre-surgical treatment assessment and planning

Treatment planning is a multistep process where clinical and imaging information are utilized to optimize treatment options in cost-effective fashion. The principle of using 3D printing is essentially based on preparing a 3D model of the targeted patient

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anatomy. The 3D model is given to the surgeon for precise planning of the surgical operation besides the cross-sectional imaging and/or, using customized prosthetics models according to the patient-specific anatomy [6,7]. This would allow a deep understanding of the complex anatomy of each different case. One of the 3D distinctive features is its ability to decide accurately the size of the prostheses components before the implantation [4,8].

For example, various treatment options were provided to optimize orthognathic surgical intervention in treating a patient presented with class 3 malocclusion with anterior open bite. He had concerns about his chewing ability and appearance as shown in Fig. 2.1.

It was not easy to anticipate ideal treatment to be followed based on normal 2D X-ray. Hence, 3D planning was conducted through optimizing bi-maxillary jaw surgery and predicting his virtual appearance post-surgery, which in result helped the patient to appreciate the treatment to be provided and eased his consent (Fig. 2.2). This is one of the established benefits of pre-operative planning [9–11].

Treatment planning can play significant role in major trauma centers where cases need urgent intervention such as severe road traffic accidents and falls. Virtual 3D planning and 3D model can aid to understand fractures present, fracture lines, fractured bones, and missing bones, through reconstructing and reassembling bone fragments together. Clear example would be gunshot injury where patient committed failed suicidal attempt and resulted in pan-facial comminuted fractures (Fig. 2.3). His 2D X-ray showed extensive facial gunshot injury with associated fractures and hematoma. There was a left-sided Le Fort 3 and right Le Fort 2 fracture. Initial planning aimed to stabilize the patient and replace fractured bones for fixation. Segmenting patient CT scan showed missing bone segments of his mandible; hence the remaining

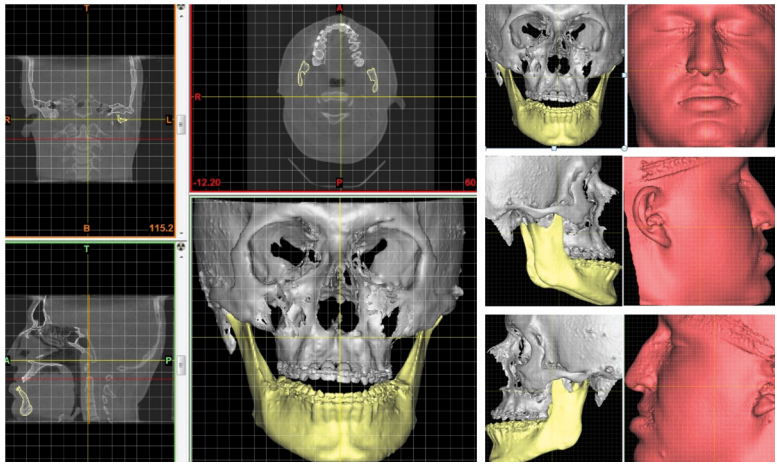

Figure 2.1 Initial segmentation of patient CT scan data showing hard and soft tissues reconstructions.

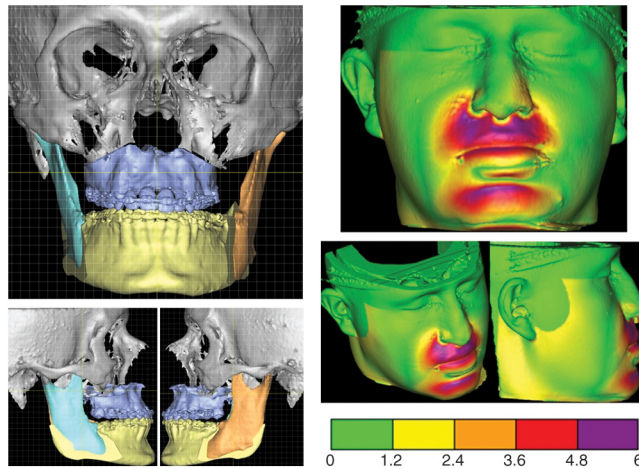

Figure 2.2 Post-operative views showing bi-maxillary surgical planning performed and resulting facial profile along with the areas (colored areas) exposed to maximum stresses (in mm).

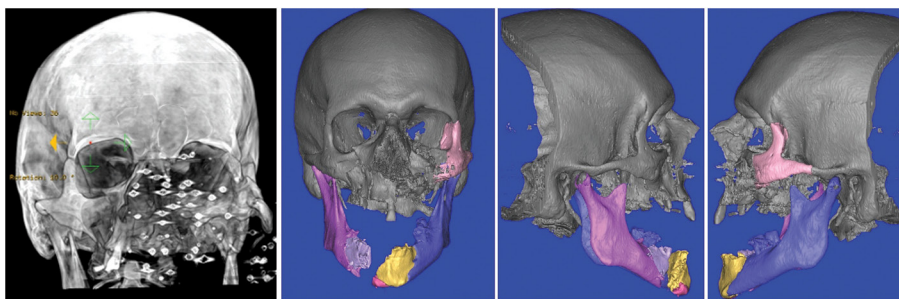

Figure 2.3 CT and segmented virtual views of patients presented with extensive displaced mandibular and maxillary fractures secondary to gunshot failed suicidal attempt. The majority of gunshot pellets are seen in the region of the left maxillary sinus and ethmoidal air cells as well as the soft tissues overlying the left inferior-lateral aspect of the mandible.

bones were assembled to normal locations and virtually fixed with bars running across the mandible. The mandible was printed, and a plate was bent to the shape of the mandible and then surgically fixed into place. Same was performed to the left orbital bones, but a biocompatible guide was produced from clear polymethylmethaacrylate which aided in bringing fractured bones together into correct assembly when plated together (Fig. 2.4).

The above examples confirm the advantages of surgical planning across medical disciplines and can be summed as saving time in the operating room (OR); assisting in reducing post-operative complications [8] and subsequently its postoperative stays;

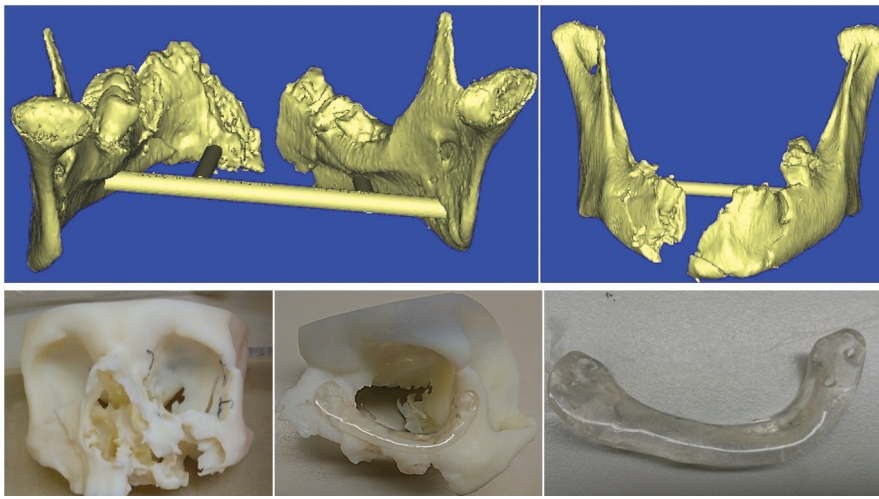

Figure 2.4 Mandible reassembled virtually showing missing anterior segment and bars joining mandibular fragments together. Bottom row shows the left orbital bones fractures which were assembled and a clear guide recorded their new assembly which was used at time of surgery.

assisting to decrease rates of revisit intervention and lastly, reducing healthcare costs [1,7,12–14].

2.3 Customized medical implants

One of the main contributions of the 3DP is reducing the costs through the customization of surgical tools and prostheses. Historically, manufacturing a lot of prostheses for a long time with limited number of sizes and reduced costs had some negative effects. For example, the levels of patients' anxiety increased as well as the quality of the produced hardware decreased [12]. Prostheses have been enabled from taking full advantage from the modeling capabilities of 3D printing. The use of custom implants for dental and maxillofacial reconstructions is widely accepted [11,15–18]. Having a pre-formed implant proved to increase surgeon's confidence, reduces surgery times and improves aesthetic outcome especially in complex cases where major parts need to be reconstructed [11,17,18]. Following case shows a young patient's skull who had multiple meningioma over years of treatment resulting in losing major bones of her skull (Fig. 2.5).

A cranioplasty implant was manufactured from medical grade titanium following conventional procedure of manual swaging which was sterilized and sent for surgery. The plate was fixed in place restoring missing skull bones and providing protection to brain (Fig. 2.6). The whole surgery took less than an hour and appearance noticeably improved post-surgery.

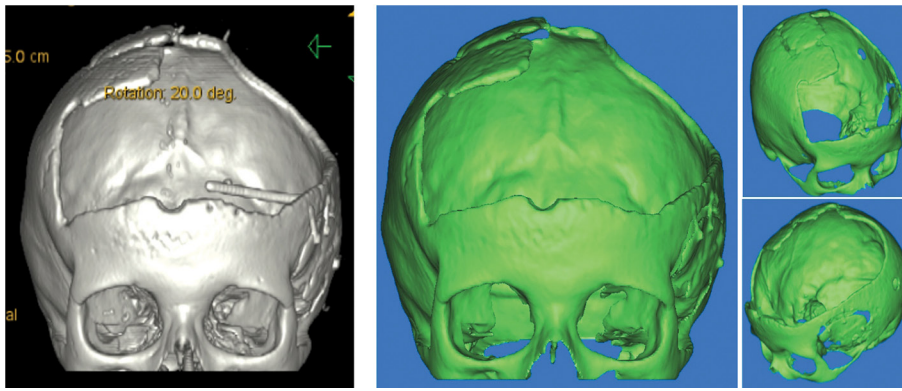

Figure 2.5 CT scan and segmented views of the skull showing the extent of bone to be restored.

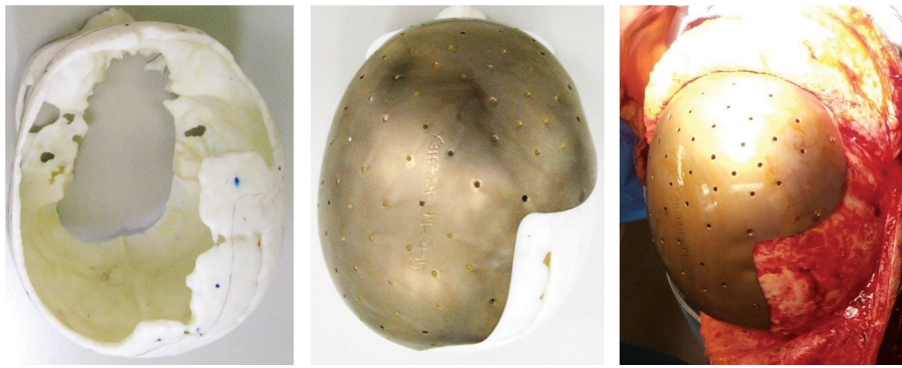

Figure 2.6 Images showing 3D printed model of the defect to be reconstructed, which was used to customize the titanium plate that used in surgery to replace missing bones.

Such customized implants become helpful in tumor resection or trauma surgery where the unwanted pathology is resected following cutting guide based on careful virtual planning and resultant defect is reconstructed with a pre-fabricated implant at the same surgery (Fig. 2.7).

Customization can be extended toward treating young children of congenital deformities. It proved helpful in prosthetic reconstruction of missing facial parts such as ears as it enables the accurate positioning of osseointegrated implants in ideal bone locations free of air cells. These implants will hold prosthetic ear in place which is a mirror image of the contra-lateral exiting ear reproduced by means of 3D virtual design and duplication as shown in Fig. 2.8. Such a procedure is already in practice and proves to save time and provide prosthetics of superior quality in terms of shape and skin color reproduction [17–19].

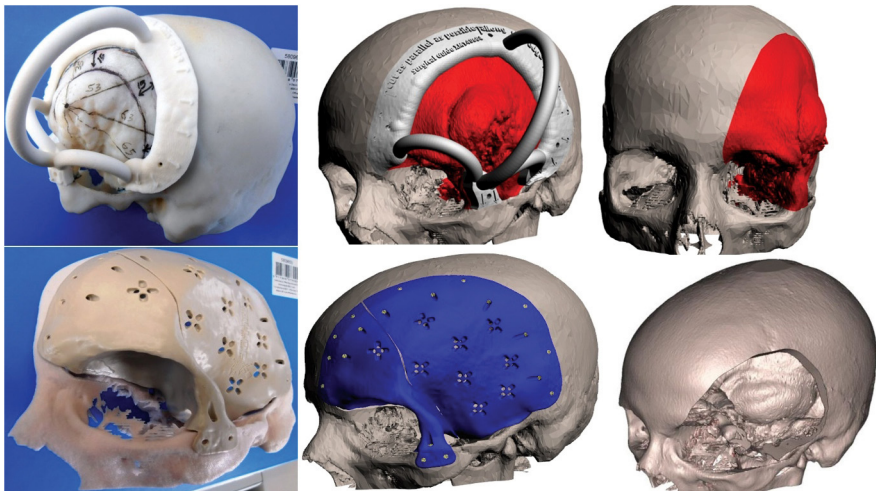

Figure 2.7 A meningioma tumor involving left parietal, temporal, zygomatic arch and sphenoid bones was characterized using 3D planning and 3DP model. Treatment planning started by virtual planning of resection translated by 3DP cutting guide and defect reconstructed by two-part 3D milled PEEK implant.

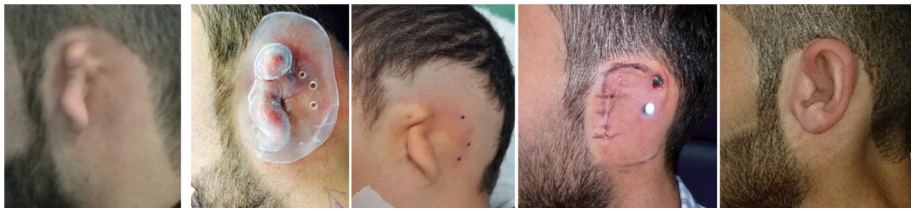

Figure 2.8 Left side implant retained ear prosthesis manufactured following 3D planning of implant positions which is then translated by 3DP implant guide to patient defect side where two implants were inserted and used subsequently in retaining a mirror image of patient's right ear.

2.4 For medical and patient education purposes

This non-clinical use of 3DP proved effective at different levels in terms of improving the performance of the learners; motivating the trainees by enhancing their self-efficacy in different domains; the fact that 3DP models are safe and reproducible when it comes to cadaver dissection; various physiologic and pathologic anatomy can be modeled from a large dataset of images; and lastly 3D models can be easily shared among different institutions particularly, those which lack resources [20–22]. Aside from these advantages, 3D printing is still unable to reproduce tiny details (e.g. small nerve braches and microstructure) [20–23].

In order to understand in-depth, complex, and management of a complicated case, physicians of different specialties can share 3D printing reproduction of specific anatomical tissue or structure. Furthermore, utilizing some features of a 3D solid and graspable object, such as the transparency and the use of the detachable parts, would enable physicians from seeing the inner structure which in turn would affect positively in approaching the views of the professionals with regard to the assessment of the 2D conventional images.

3D models as solid and graspable objects have been considered as great tools that support physicians to explain to their patients the medical conditions visually [24]. For example, showing a patient a 3D model would facilitate the task of the physician in explaining the case in hand. It would provide the patient with full understanding to the case. As a result, an approval on the proposed treatment can be obtained as well as the possibility of medico-legal controversies might reduce [24–27]. Previously mentioned cases have an element where patient is shown the 3D planning and printed model of treatment to be achieved and its risks.

2.5 Bioprinting and modeling

Other clinical uses of 3DP include bioprinting and modeling of implantable tissues which are considered among the very recent and advanced uses of the 3D printing. For example, patients who are suffering from burn injuries can get full advantage from 3D printing of synthetic skin for transplanting [28]. Furthermore, producers of cosmetics, chemicals, and pharmaceuticals are also able to test their products through the use of the 3D printing techniques. Amazingly, 3D printing has enabled physicians from reproducing heart valves through the use of a combination of cells and biomaterials to control the valves stiffness [29]. Likewise, 3D printing is used to reproduce human ears through making models filled with gel. Furthermore, while the customized synthetic organs through the 3D printing would give a chance to secure life via reducing the number of patients who need transplantation, bioprinted organs might be used by pharmaceutical industries as an alternative of animal models which aim to examine the toxicity of new drugs in the future [30–32].

2.6 Conclusions

3D printing is one of the most innovative technologies that has proven its potentials to make revolution in the clinical field. This was demonstrated through case examples shown earlier. It improved the practice of medicine and healthcare as technology became affordable, accessible, and easy to follow. Added to that, the continuous development of the printers increased the level of controlling safety while printing biomaterials.

Taking into account the 3D printing in the medical field, one should think outside the norm for improving and developing health care as it enables more treatments to be

conducted and keeps risks to the minimum, along with improving treatment outcome at reduced operative time. 3D printing provides a good example on the innovative and effective technologies that has many advantages. Furthermore, 3D printing is liable to find an application according to the case. However, this should consider the updated and current legislation to guarantee its correct and accurate use.

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Part 1

3D Printing Techniques for Augmenting Medicine

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3D printing techniques in medicine and surgery

3

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Chapter outline

3.1 Introduction

From its current context, 3D printing is the process by which a 3D digital design is changed into a component by depositing material using additive processing. As a medical manufacturing process, components are made layer-by-layer from a wide range of polymers and metallic materials. These are formulated as liquid resin, solid, biological and powder forms. Because of its flexible nature, an array of metallic, polymer and composite materials can be produced on demand. With the right degree of skills, this makes seemly complex geometries and structures easy to make.

As of writing, 3D printing is a swiftly evolving technology consisting of many different methods for the fabrication of a new generation of healthcare products. The most important aspect of this technology is that due to its flexible manufacturing

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nature, it is capable of making parts that are scalable and which are able to aid in numerous medical procedures. It also has the potential to produce a wide range of healthcare products. Currently research extends toward the realms of manufacturing complex moving components from a range of innovative and functional biomaterials.

Current medical applications for 3D printing are expanding at a fast rate so that they will be able to provide customized treatments for patients [1]. The medical uses of 3D printing, both actual and potential, can be categorized into a series of key themes. These are:

- 3D tissue and organ biofabrication;
- Fabrication of customized prosthetics,
- Patient-specific implants,
- Anatomical models,
- Pharmaceutical research and
- Surgical instruments and tools

These key areas are of direct importance to many healthcare practitioners. The direct application of 3D printing in medicine provides significant benefits, including:

- The fabrication of personalization/ patient-specific medical products,
- Fabrication of drugs,
- The production of custom equipment,
- Cost-effectiveness design and fabrication,
- Rapid fabrication,
- Opensource design and fabrication of medical devices and systems and
- Enhanced collaboration between interdisciplinary teams.

In this chapter, we consider the practical applications of 3D printing technology and the techniques that can be used to fabricate a wide range of medical related devices.

3.2 3D printing from the beginning

In the Fall of 2012, I was first introduced to 3D printing; at the time, it was very new. Our first machine was made out of wood, but it was beautiful, easy to use and quick. It was a Makerbot Replicator 3D printer as shown in Fig. 3.1. It was very advanced

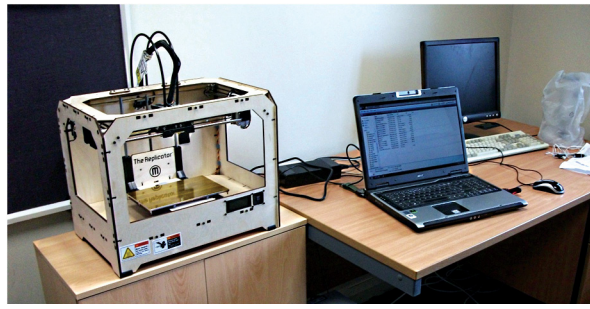

Figure 3.1 The authors first 3D printer, a Markerbot Replicator in his office used to fabricate 3D Printed models for surgical planning.

for the time, featuring two extruders, it could print two materials simultaneously. This Fused Deposition Modelling (FDM) machine was a great addition to the work that we were doing and one that was both fun to use and easy to build accurate 3D models with.

Our initial plan was to generate 3D printed medical models for preoperative planning. This soon extended to the fabrication of a range of healthcare devices. Even eight years later, our first 3D printer is still hard at work manufacturing a range of 3D printed parts for healthcare professionals.

It was soon found that when medical practitioners were introduced to 3D printing new ideas were developed and before long there was a synergy between traditional engineering and the healthcare sector. Now it has become a rapidly evolving technology consisting of many different methods for fabricating a new generation of advanced components and structures.

The first medical models generated got better after each printing as we learned how to both use and optimize the machine. Within a week we had gone from first taking the device out of the box to the fabrication of the first preoperative surgical models as shown in Fig. 3.2. This was used to plan plastic surgical procedures.

Colleagues were amazed, fascinated and curious about how this technology worked. So, we began to provide demonstrations. The Makerbot Replicator was transported from hospital to hospital, so medics could see first-hand the benefits of 3D Printing technology. Within a month, we started to fabricate large and more complex models, made using ever more exotic and interesting materials, including:

- Dental models: 3D printed from polylactic acid (PLA) (Fig. 3.3A)
- Craniofacial models: 3D printed from PEG (Fig. 3.3B)
- Soft tissue models: 3D printed from polyurethane (Fig. 3.3C)
- Bone models: 3D printed from Nylon 645 (Fig. 3.3D)

We continued experimenting and developing the technology. Every 3D print that we produced became quicker, more efficient, and accurate. There was no stopping the revolution that this technology gave us.

Over the following 8 years, our research advanced from creating simple small models to complex larger models. It progressed toward creating advanced prosthetics,

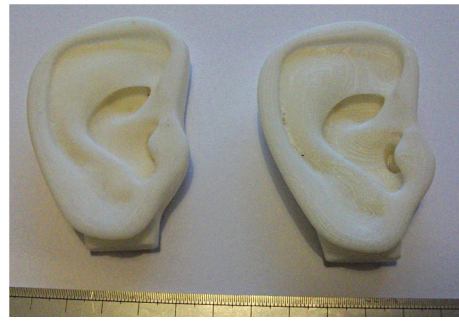

Figure 3.2 3D printed surgical models of the ears. Each was 3D Printed from Poly-Lactic Acid (PLA) and each small model took 2 h to 3D print.

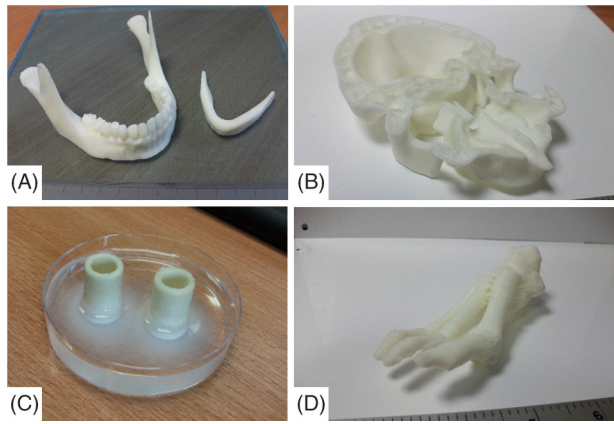

Figure 3.3 3D printed models (A) Dental models - 3D Printed from PLA, (B) Craniofacial models - 3D Printed from PEG, (C) Soft tissue models - 3D Printed from Polyurethane, and (D) Bone models - 3D Printed from Nylon 645.

surgical instruments, and medical devices [2]. Currently our research focuses on 3D bioprinting. We produced the first 3D bioprinters for hospital use, and the continuing theme is that innovation has progressed the field.

When 3D printing meets biological materials, then this has the potential to create monumental change, that is, shifting the current status quo to a new frontier. It is only through learning the process of 3D printing on inert materials that we are then able to create more complex biological systems [3].

So how is this achieved? Well, it is complex. But this chapter shows a chronology through the 3D printing technologies, the ideas, concepts, and processes. It is important to consider them all. For healthcare, there is no true one size fits all. However, experimentation is exciting, healthcare provides are an exciting application for this technology. And when these two domains collide, then we get a true shift in technology and ultimately an evolution in this technology [4–6]. Over the past number of years, we have experimented with 3D printers and built our own 3D printers and 3D bioprinters. This adaptive approach toward developing new equipment and technology has resulted in both success as well as failure. This chapter details the techniques that are required to 3D print a range of components for medicine and surgery.

3.3 The 3D printing process

We start first with the 3D printing process and how it works. With 3D printing in medicine and surgery, it starts with the patient. Considering the patient need is of paramount importance. As a result, we first need a model that can be 3D printed. This is generated from a wide range of sources, DICOM 2 standard CT scans. Conventional photos are joined together to form 3D model structure. Traditional computer aided design, 3D laser scan through and sculpt a structure using software tools such

Figure 3.4 The process through which 3D Printed parts are created from patient data.

as blender. These are a good means toward the formation to create designs and derive solutions [7]. Fig. 3.4 shows the general principle for the creation of 3D printed physical structures from a 3D design.

It is done through the control of numerical data and physical materials' properties; by then the accuracy of medical objects could be increased significantly. The strength of 3D Printing in medicine is that complex geometries can be produced from a range of advanced materials. As a result, these structures can be obtained of any shape or geometry, produced from an accurate 3D model source [8]. When considering the advantages of 3D printing for producing a design for the purpose of testing, then it is possible to consolidate many individual parts of an assembly into a single and complex medical part. The advantage of this approach is that it eliminates part numbers, inventory, assembly, labor, and inspection, which is where the power of 3D printing remains [9].

During the investigation of this book, it has been revealed that healthcare professionals have a significant interest in the applications of 3D printing technology. This is for a wide range of procedures of varying degrees of complexity [10–12]. At the high end, is the fabrication of transplantable structures, and qualifying these structures for regular use is becoming of critical importance.

In order to rebuild hardwearing joints, we need to integrate suitable polymers in order to aid stability and couple this with a durable permanent implant. This has been found in a 3D printable material ''Nylon 645" which at 320MPa UTS is pound for pound stronger than titanium alloy. Fig. 3.5 shows final implant created of a meniscus disc structure 3D printing using Nylon 645. The complex materials are capable of being transplanted for a wide range of biological substitute components.

The two most critical factors to control carefully in 3D printing are the layer resolution and the deposition of the exterior shell [13–15]. It is these two significant properties which control the edge and surface accuracy. This formation is shown in Fig. 3.6, that is, the shell is deposited in FDM and 3D bioprinting processes first. This ensures that the infill structure can be produced accurately.

If we take this one step further, then we can fabricate custom 3D printed functionalized parts so that they comply with ISO 10993 biocompatibility standards. Future applications include:

- Artificial fillers
- *Tubing*

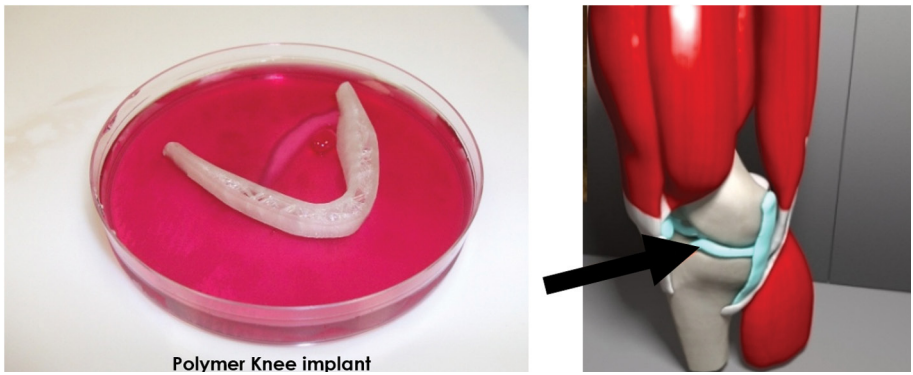

Figure 3.5 Prototype cartilage joint replacement implant 3D Printing from Nylon 645.

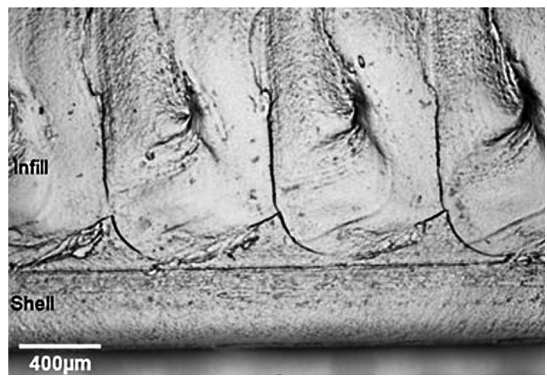

Figure 3.6 The two most critical factors to control carefully in 3D Printing are the layer resolution and the deposition of the exterior shell. It is these two significant properties which controls the edge and surface accuracy.

- *Catheters*
- *Implanted drug delivery devices*

As shown in Fig. 3.7 is the formation of the first stage in the 3D printing process. Liquid polymers, including two-part polymer systems such as PDMS can be 3D printed to form complex structures. By combining composite materials, we can make hybrid implants which are patient specific. With future development, we plan to integrate further advanced polymers that can potentially be used to produce a moving part; this could in the first instance be heart valves.

The high potential for medical 3D printing is that it is possible for all medical professionals to design and produce or redesign a range of components quickly and cheaply. Today, automated 3D printers can directly produce functional parts in small production quantities.

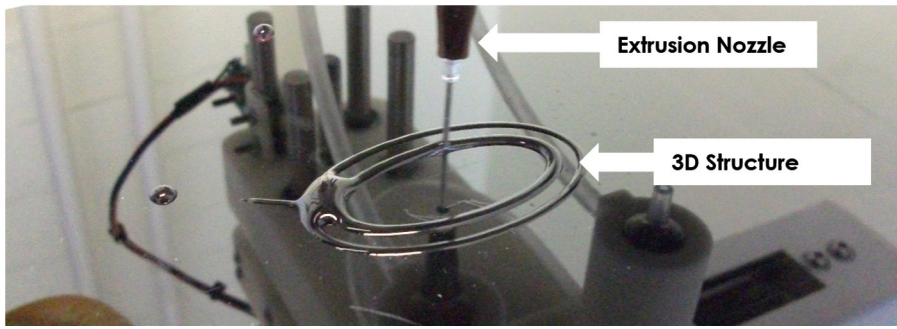

Figure 3.7 The critical first layer of the 3D Printing process. Here two-part PDMS is being 3D Printed to form a transplantable graft.

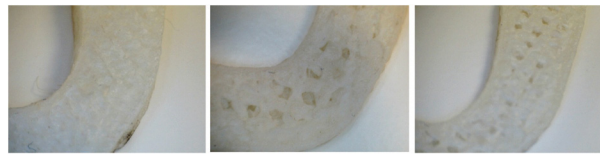

Figure 3.8 Photographs taken of 3D Printed implants with different levels of infill.

By being able to 3D print medicine objects with hollow structures as shown in Fig. 3.8, then the component can have a thin outer shell which includes internal lattice structures instead of solid material throughout [16,17]. This substantially reduces the amount of material used, weight, and production time. It is also possible to redesign parts using component optimization methods. The amount of material and time can as a result be reduced by up to 90% using this technique.

This is also of critical importance to the 3D printing process as the voids between the materials can be filled with other materials, medicines, and growth factors [18]. The current technical challenges include system reliability and process repeatability, especially when using 3D printing for actual component manufacturing. The current limitation in build speed and maximum part size are challenges which can be addressed by using different 3D printing technologies and materials [19]. This is being solved by developing new systems with larger build volumes and whole layer at a time deposition technologies, which will allow an increase in throughput. The current healthcare opportunity of 3D printing is the ability to produce a wide range of objects on demand with sophisticated internal structures, such and microchannels, actuators, and integrated moving components [20–22]. This will allow for the fabrication of advanced integrated implants.

Fig. 3.9 shows the fabrication of an implantable structure that provides the best compromise between surgical and technical domains. Here the complete ear structure

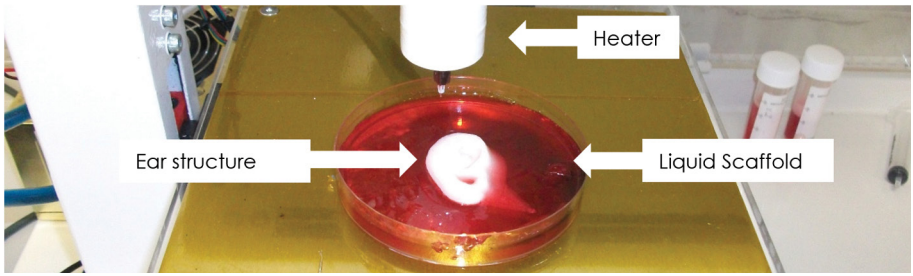

Figure 3.9 3D Printing of a complete polymetric implant of the ear of a child. The structure can later be trimmed to suit the needs of the surgeon.

is fabricated from PDMS where the surgeon can sculpt the structure to suit their requirements. In the context, 3D Printing is used as a method to augment surgery and is a good alternative to conventional surgical procedures [23]. From a medical context, the key advantages of 3D printing point that it:

- improves communication between patient and the medical practitioner,
- shortens development design cycles,
- offers the opportunity of patient-specific medicine,
- improves surgical accuracy,
- minimizes costly mistakes,
- triggers new and unexpected ideas,
- drives innovation and quality, and
- improves collaboration between teams and other disciplines.

3D printing processes are as a result a more efficient means toward creating physical components which do not require any special processed materials. It is also inherently superior in comparison with traditional subtractive or formative manufacturing processes. After the process of developing the design, due to its high precision nature, the wide range of materials are available and the unlimited complexity of parts allow autonomous manufacturing [24].

The earlier adopters of 3D printing within the medical discipline were plastic surgeons who required models to plan procedures. This low volume form of 3D printing was also of high value. As 3D printing systems speed increased, equipment and material costs reduced, more novel materials become available, and new manufacturing applications have emerged.

There are five key methods which 3D printing processes used in healthcare that can be classified as (1) Fused Deposition Manufacturing, (2) Stereo Lithography, (3) Selective Laser Sintering, (4) Binder jetting, and (5) 3D Bioprinting. They can also be classified broadly by the initial form of its material which a prototype part is built with. In this manner, 3D printing systems can also be categorized into (1) liquid based, (2) solid based, (3) bio-gel based, and (4) powder based. 3D printing processes can also belong to the (1) Melting and Solidifying, (2) Fusing method, (3) Extrusion, or (4) Cutting and Joining technologies.

3.4 3D-printing process from patient to process

3D printable models are either created with a computer aided design software or data are acquired via, CT scanning as shown in Fig. 3.10 or 3D laser scanning. 3D laser scanning is a process of analyzing and collecting digital data on the external shape and appearance of a real object.

As shown in Fig. 3.11 the highest quality method for rebuilding a 3D architecture is by acquisition of data from computed tomography (CT) data. The standard format used to represent these data is the digital imaging and communications in medicine (DICOM) 2.0 standard. During the scanning process, data should be gathered in 0.5-mm sections.

The information from each plane is put together to provide a volumetric image of the structure. Later these data are used to determine the location of that anatomical structure. Three-dimensional CT image post-processing involves generating volumetric by stacking each scanned section on top of each other. Data separation of relevant

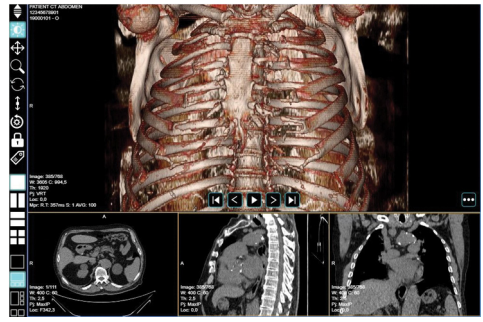

Figure 3.10 The processing of DICOM 2 sections to form a 3D anatomical model that can be used to rebuild a 3D structure: (A) Abdomen rebuild and (B) Renal structure rebuild.

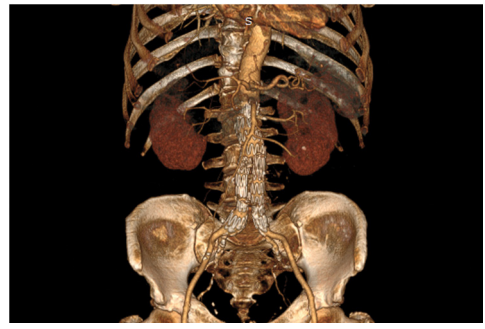

Figure 3.11 The process through which CT scans are converted into a 3D Model: (A) scan of a patient using the DICOM 2 standard, (B) isolation of the areas of interest, and (C) rebuild of the sections to create a 3D model geometry.

tissue soft and bony structures is performed from this data volume. This is determined by a combination of threshold and exclusion techniques of nonrelevant tissue. From this, a three-dimensional model is constructed using edge-detection image processing.

Volume surface rendering, transparency, colors, and shading are all used to allow a better representation of the volume to be shown in a single image. Segmentation, which is an automatic procedure, is used to initially remove the unwanted structures from the images. In order to convert these data into a format that a 3D printer can use, the OsiriX software tool is used to produce mesh of points from CT images [25]. This surface render in its exported format was manipulated and made compatible for 3D printing.

The files are firstly exported as obj files and opened in Blender software which allows for further mesh manipulation. The software allows for holes in the structure to be closed in the meshes and for any remaining abnormalities or artifact produced in the scans to be removed. These files are then exported as standard triangulation language (STL) file format which was then converted into the toolpaths which was used to 3D print the model.

In accordance with these data, three-dimensional models of the scanned object can then be 3D printed. Starting from a computer aided design, this is converted to a standard Tessellation language file. Following the acquisition of the 3D structure, the next phase is based upon processing the geometry using an integrated slicer. This converts the model into a series of layers and produces a G-code file. G-code is ultimately the set of instructions that control the 3D printer. These are specific to the type of 3D printing process at the model on 3D printer.

For the medical professional, there are numerous integrated slicers that can be used depending on the level of control required. ReplicatorG is an opensource software tool that allows for the generation of G-code, it allows for the control of essential 3D printing parameters. Further slicing engines include Slic3r, and Cura, and these convert a 3D geometry into G-code and account for process parameters: layer height, print speed, providing integrated scaffolds and allowing precise control of the print temperature. Using extrusion processes then complex components can be produced to a ±25 μm degree of accuracy. The user interfaces of Slic3r and Cura are shown in Fig. 3.12.

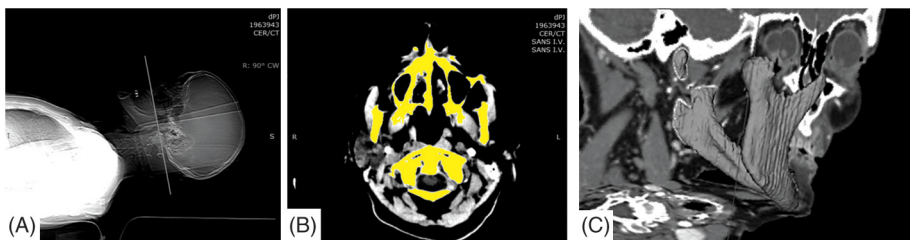

Figure 3.12 Images show on two different G-Code generation engines (left) Slic3r and (right) Cura version 3. Both generation of the same code (G-Code) that the 3D printer executes to produce a 3D tool path.

The 3D printer executes the G-code instructions one line at a time to precisely deposit successive layers of liquid, powder, paper, or sheet material to build the model from a series of these sliced cross sections. These layers, which correspond to the cross sections of the design fuse to create the final shape. The primary advantage is that during the 3D printing process that it is automated. By controlling the formation of internal scaffold structures, then it allows for almost any shape or geometric feature to be produced.

Through the selection of the correct process settings, then a wide range of complex geometries can be fabricated in 3D. The print resolution is the layer thickness that is controlled during the printing process to form a high definition structure. A balance is made between layer resolution and the time that it takes to build the model. Typical layer thickness is around 100 μm (250 DPI) are standard, however, high-end systems can print layers as thin as 15 μm (1600 DPI). Of secondary importance is the X–Y resolution that forms each layer. This is usually in dots per inch (dpi) or micrometers (μm).

Modification of 3D models can also be undertaken to include holes, fixings and mechanical connectors. A typical example of this is shown in Fig. 3.13 in which a dental structure has been designed to incorporate a hole for a screw fitting. By designing and printing the model complete with such structures reduced the time to make such components to typically a few hours. This depends on the type of 3D printing machine used and the size and number of models being produced simultaneously.

There are numerous 3D printing systems that are capable of using multiple materials during the fabrication of a part. Some are able to print in multiple colors, different polymetric materials and even encompass dissolvable scaffolds made from PVA [26]. These scaffolds are removable or dissolvable upon completion of the print and are used to support overhanging features during construction.

It is through the control of process parameters during the 3D printing process that we can control the formations within the structure at a microscale. As shown in Fig. 3.14, three-dimensionally bioprinted calcium phosphate bone composites can display optimum fusing properties. When the material is deposited at the correct temperature then it becomes workable enough to form precise structures.

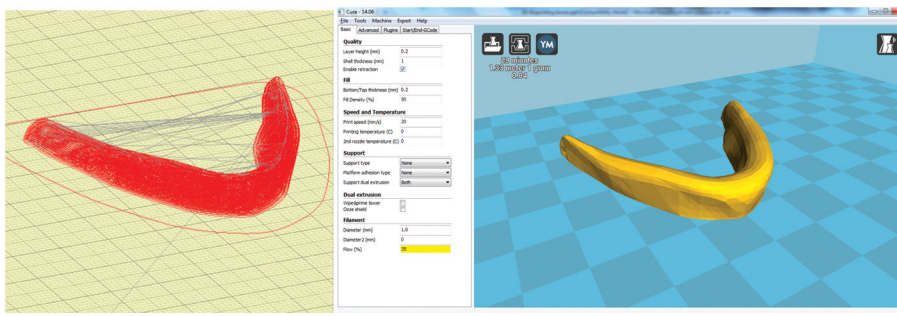

Figure 3.13 The design and geometry of a hollow channel feature 3D printed within a small bone structure.

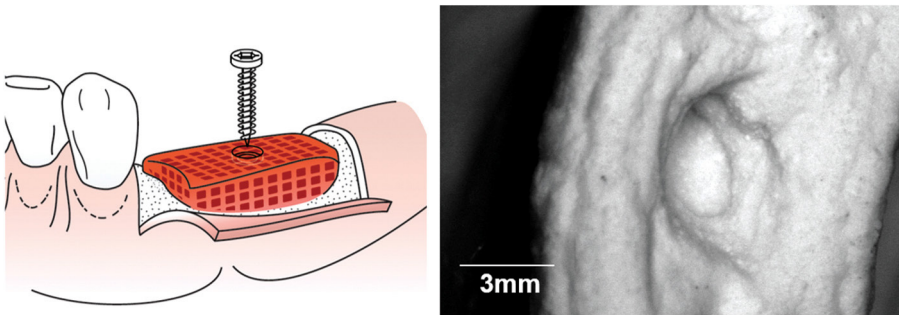

Figure 3.14 Micrographs of the fine infill detail generated by 3D printing polycaprolactone 3D printing filament. Here a fine tip FDM 3D printer has been used to produce a hexagonal infill pattern. The G-Code has been generated using Replicator G software.

It takes under one hour to be able to print a small 10 mm^3 polycaprolactone (PCL)-based bone structure with micro-trabecular features. Realistically this could be carried out before a procedure so that it is ready when needed. Because of this, then it can be ensured that 3D printed bone composite materials are generated using the correct process settings to ensure a smooth surface structure is produced with no stress raising features or cracks resulting from post-machining processes.

By controlling the concentrations of the different constituent elements then the structures mechanical and material properties can be altered.

One of the most exciting and closely watched new uses of 3D printing is for producing patient-specific 3D printed implants, such as for cartilage joint replacement [27]. These are often more difficult than bone replacements as the part must accurately conform to an existing internal bone structure and be pliable enough to conform to unusual mounting methods. They must be inherently strong to keep the joint from becoming misaligned by stress, and most important, provide a long-term slippery surface to the biological mating surface.

3.5 3D printable materials

There are many materials that can be 3D printed; there are currently over 20 main families of material that can be used. However, a number of important factors that need to be understood in order to select the most appropriate material. This includes type, minimum thickness, minimum layer height, and surface finish.

3.5.1 Acrylonitrile butadiene styrene

ABS filament has proven to be the most popular 3D printing material for generating 3D medical models. It is been used commonly over the past 10 years for FDM printing due to its high-quality surface properties. It is resistance to damage, it can be

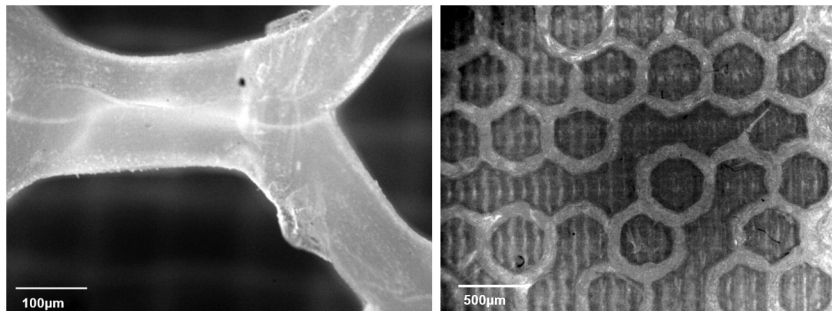

Figure 3.15 3D Printed ABS structures on a home entry-level 3D Printer (left) the production of a novel syringe concept and (right) the partial fabrication of a tumor structure 3D-Printed using a 1:1 scale.

drilled, used to manufacture surgical tools and guides and practice models as shown in Fig. 3.15. It is strong and slightly flexible, which makes it a good material for 3D printing. In addition, ABS filament has a 32 MPa of tensile strength and can be easily extruded using a wide range of 3D printers.

The material is purchased in either 3.0 or 1.75 mm diameter filament reels of 1 kg up to 2.2 kg. The extrusion temperature range of ABS is 220–230°C and a heated build platform is required that needs to be 115°C. ABS combines strength together with being lightweight. Models can also be drilled and handled many times. It is a low-cost material, but due to the fact that it is petroleum-based means that it creates fumes and fine particles can be expelled. The 3D printer therefore needs to be used within a well-ventilated room.

3.5.2 Polylactic acid

PLA filament has a wide range of 3D printing in medical applications, as it also offers biodegradability. Made by polymerizing sugar cane and potato starch, it has a low-toxicity. PLA has the ability to degrade into lactic acid in the body and due to this property, it has been used in medical suturing and surgical implants. Surgically implanted screws, pins, rods or mesh naturally break down in the body between 6 and 24 months. However, there have been a number of side effects resulting from implantation because of foreign body reactions [26]. Therefore, it should not be used for surgical transplantation (Fig. 3.16).

PLA is a key material for printing using an office-based 3D printer and is environment-sustainable. Because PLA only shrinks by 1.5% on cooling so there is no requirement for a heated bed. PLA can be procured in both 1.75 and 3 mm diameter filaments. There are also further derivative PLA materials that are crystal clear, soft, and impact resistant and is great for the manufacture of a wide range of 3D printed medical models and surgical instruments. PLA requires a 3D print temperature range: 195–215°C.

PLA is the best material for medical professionals who want to start printing a range of devices, models, and instruments. Because of its affinity to attract water it can

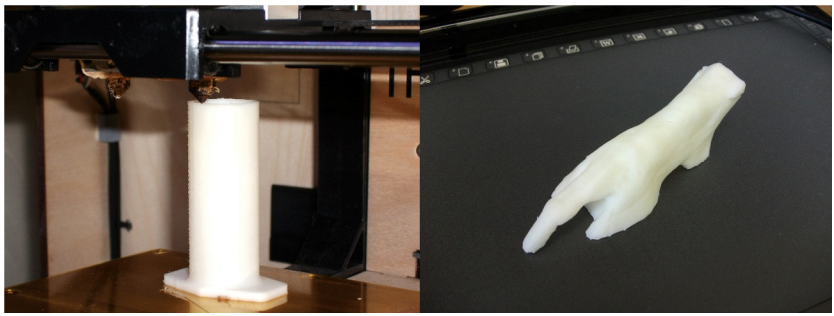

Figure 3.16 3D Printed PLA in the shape of atlas vertebra. This model features a rough surface as a result of the layer resolution. This model was 3D printed using a 1mm layer height.

Figure 3.17 3D printed experimental water-soluble structure that is being testing to determine structural degradation.

become difficult to print with as it gets older. It is important to ensure PLA is stored in a cool dry place when it is not being used.

3.5.3 Polylvinyl alcohol

PVA is a special plastic that is water-soluble it is both non-toxic and biodegradable. PVA is easy to 3D print and they make good support during a printing process for those models with overhangs that is impossible to print without support. This is achieved using dual extrusion 3D printing (Fig. 3.17).

PVA prints using a print temperature range of 170–190°C and becomes more soluble in water depending on the temperature at which it is 3D printed. One consideration that needs to be made is that it attracts water and needs to be dried well before it is used. Where there is a medical need for rapid solubility, then PVA can be used safely for such applications.

3.5.4 Polyethylene terephthalate

PET is a food safe material that is both stable and biocompatible. It can be used for a range of medical applications including sutures, bone grafts, and vascular grafts as

Figure 3.18 3D printed experimental vascular grafts 3D printed from PET.

shown in Fig. 3.18. It has useful medical properties including hardness; stiffness; biochemical and long-term dimensional stability. PET has many promising biomedical applications due to the presence of hydrophobic aromatic groups with high crystallinity it restricts hydrolytic breakdown. However, due to the absence of bioactiveness it is not suitable for tissue engineering.

PET filament in its original state is crystal clear; however, if it is overheated, then this causes it to become cloudy in appearance. As a result, PET needs to be allowed to cool slowly so a heated build platform is required. Components 3D printed from PET can be machined and polished to form smooth surfaces. Because of its thermal sensitivity, there is a narrow print temperature range of 200–210°C if a clear finish is required. PET filament is ideal for medical structures that require flexibility and impact resistance.

3.5.5 Polyethylene terephthalate glycol

Polyethylene terephthalate is glycol modified for extra durability. This is a very tough material that is extremely durable and can be used for a wide range of medical experiments. PETG filament offers durability and impact-resistance that is superior to PET as the latter tends to become hazy and brittle when overheated. The addition of glycol removes these limitations giving PETG filament the following properties: low shrinkage, no warping, and strong but not brittle (Fig. 3.19).

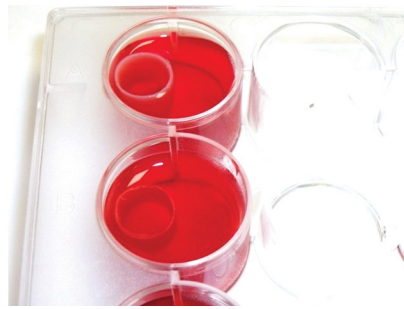

Figure 3.19 3D printed ear structure being washed before being validated.

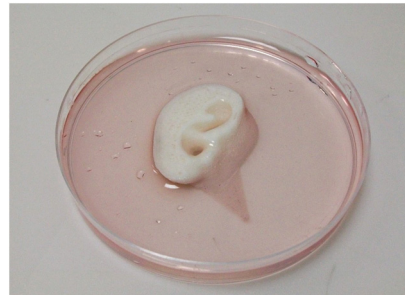

Figure 3.20 3D printed PETT structure produces as a four-layer structure with a complex top geometric structure.

PETG has a wider print temperature range of 220–245°C and it best used for 3D printing medical applications that are subject to sudden or sustained stress. It is particularly good for a range of surgical instruments. This also has the advantage of long-term biocompatibility. The material has a high strength and some flexibility, and it is also not brittle or prone to warp when stressed. PETG does not absorb water or moisture from the air, does not degrade in liquid.

With all of its advantages, PETG is not easy to use and requires fine-tuning of bed and nozzle temperature. One observation with the materials is that is can produce thin hairs on the surface which need to be removed after 3D printing.

3.5.6 Polyethylene cotrimethylene terephthalate

PETT is colorless and crystal clear. It does not degrade and does not biodegrade. PETT is FDA approved, making it safe for direct food contact. Immediate PETT applications include medicine containers and surgical instruments (Fig. 3.20).

PETT prints well using a temperature range of between 210 and 230°C. This material has a good combination of strength, flexible, and biocompatibility. It is not brittle and does not wrap when stretched. When printing PETT then a glass-built platform is used so that its base will have a flawless finish. PETT does not absorb water or moisture from the air, and does not degrade in water. It is also FDA approved and can be used in numerous creative surgical applications.

3.5.7 High impact polystyrene

HIPS is biodegradable and has a bright white color. It has no adverse effects when it comes in close contact with biological structures. HIPS is similar to ABS as uses Limonene as a solvent during manufacture.

HIPS has a wide print temperature range of 210–250°C and is the perfect material to fabricate a range of surgical and dental instruments as shown in Fig. 3.21. These can be designed and built to suit the needs of the healthcare professional. HIPS does not warp but needs a heated build plate to allow 3D printing. One of the most useful

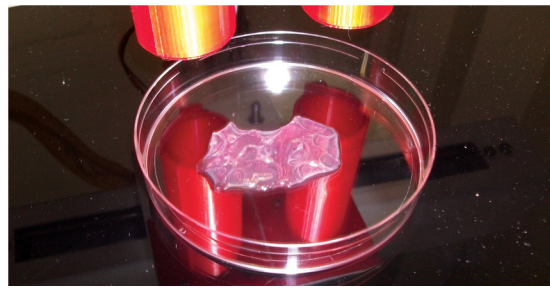

Figure 3.21 3D printed prototype dental instrument that is 3D printed from HIPS.

applications of this material is in the fabrication of prosthetic devices that require good impact resistance and long-term usage.

3.5.8 Nylon 645

Polyamide better known as Nylon is a popular synthetic polymer that is also used in many healthcare applications. Nylon 645 is the highest performance 3D printing filament for mechanical and functional parts due to its high strength. Considering Nylons' durability, it can be used in a variety of applications including implants that need to be mechanically strong as shown in Fig. 3.22.

Nylon has a higher print temperature of 250–260°C and requires an all metal printing nozzle. It is perfect for applications that require long-term wear resistance and high strength. The material itself is sensitivity to moisture and can absorb over 7% of its weight within 24 h. It therefore needs to be stored in a dry container after each use.

Figure 3.22 3D printed Nylon 645 knee implant made for mechanical assessment.

Figure 3.23 A 3D printed metallic structure placed inside a high temperature furnace.

3.5.9 Metal transfer PLA

A recent application of 3D printing technology is that of metal transfer 3D printing. In this type of filament metallic particles including (copper, aluminum, and brass) have a high concentration. Following the 3D printing process, components are placed into a high temperature furnace. The component is slowly brought up to the correct temperature required to melt away the PLA base polymer and fuse together the metallic particles; this is shown in Fig. 3.23.

Print temperature range: 195–220°C and firing is carried out in a furnace to the required fusing temperature of the metallic material. The polymer matrix subsequently melts away and the metallic particles fuse together.

This process does require increased expertise; however, the components produced are alloy structures that can be used in a wide range of medical applications. The parts produced have a high degree of durability and are subsequently permanent components. There is however a lot of fine tuning to be carried out during the printing process as every metallic filament material is different. Parts will also shrink extensively as the metallic part forms, so each component needs to be scaled up before 3D printing.

3.5.10 Carbon fiber PLA

Carbon fiber uses PLA as a matrix material and short carbon fiber to reinforce the structure. As a result of this combination, it offers excellent structural rigidity, structure, and layer adhesion. However, it is stiffer than PLA with better dimensional stability for warp-free printing, excellent layer adhesion, and easy support removal.

This material can be used to generate a range of surgical instruments as shown in Fig. 3.24, prosthetics and components that require dimensional stability and long-term strength. As the base matrix material is PLA then the print temperature range is 190–210°C which is 5°C lower than traditional PLA as the carbon fibers inside conduct heat.

There are a wide range of applications for carbon fiber-based materials where durability is required. The integration of short carbon fibers makes a significant difference to stiffness and results in only a small amount of shrinkage upon cooling, so the part produced is dimensionally accurate to the model.

Figure 3.24 3D printed carbon fiber surgical instruments.

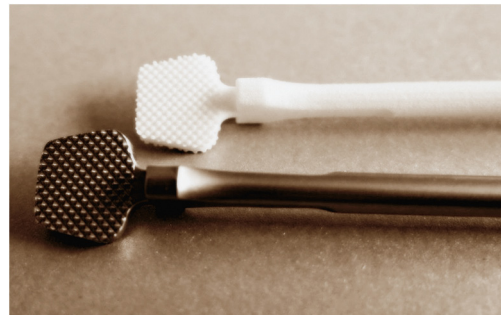

Figure 3.25 An example of a TPE model produced to illustrate the color and flexibility required for prosthetic devices.

Care must be taken during the 3D printing and the carbon fibers will abrade the internal structure of the nozzle during 3D printing. Therefore, stainless steel nozzles are required over that of traditional brass.

3.5.11 Flexible thermoplastic polyurethane

TPU filament is a class of polyurethane plastic and is perfect for uses where there are requirements for elasticity, transparency and resistance to abrasion. The TPU filament has high elastic characteristics that can be used in a wide-range of prosthetic devices that need to mimic soft tissue. Taking into account its characteristics and durability, it is a material that is good for long-term applications (Fig. 3.25).

Because of its high degree of flexibility, TPU is a good candidate from facial prosthetics and for nipple reconstruction. The material has a high elasticity and is very resistant to abrasion. It bonds to the build platform and can be 3D printed straight onto other objects. Because of its affinity to bonding between layers the objects produced are of high-quality.

3.6 Fused deposition manufacturing

FDM 3D printing is a process of making a three-dimensional object by laying down and fusing polymer materials in layers. For the purpose of medical 3D printing, this process is by far the most flexible, low cost and popular method of 3D printing. As it

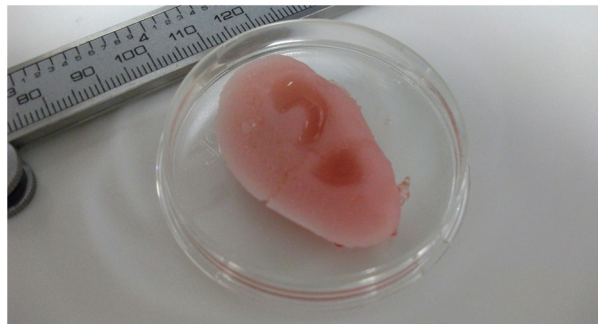

Figure 3.26 A typical FDM 3D printer with the various elements demonstrated in an FDM 3D printer.

has been seen previously, there are a wide range of FDM materials that are available as shown in Fig. 3.26.

This 3D printing process which used the extrusion of thermoplastic material is the most common method of 3D medical printing. The mass proliferation of entry-level 3D printers that are needed by the medical and healthcare sector has emerged since 2009 largely utilize a similar process, generally referred to as Freeform Fabrication (FFF). The earliest RepRap machines and all subsequent evolutions employ extrusion methodology.

The 3D printing process works by heating plastic filament to it glass transition point temperature. The polymer material is then deposited through a heated extruder called a hot-end. A technical illustration of an extruder assembly is shown in Fig. 3.27. Each layer is deposited one at a time, onto a build platform. As the layers cool, then solidify, and bond to the layer underneath to form a 3D structure.

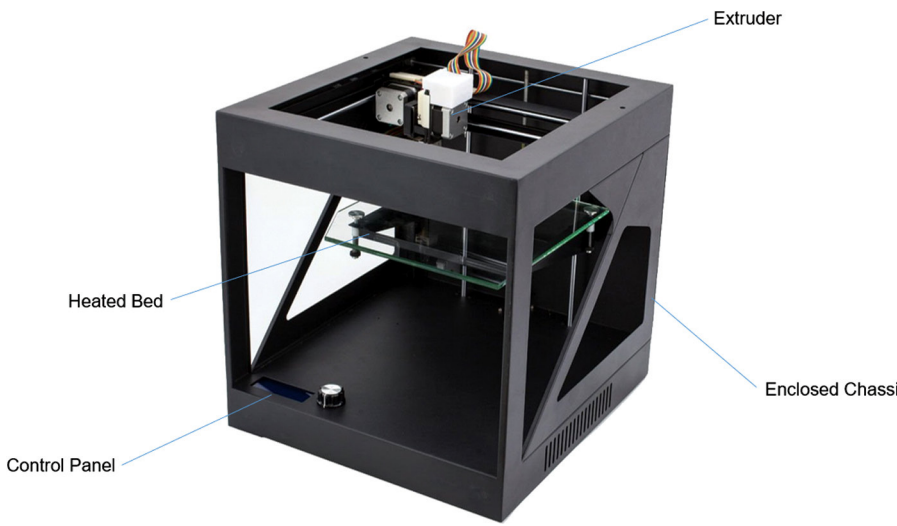

Figure 3.27 3D printed Illustration of the working principle of FDM-based 3D printing [28] .

FDM and FFF processes require removable support scaffold structures when parts are built with overhanging geometries. Here a second extruder is often used to deposit water-soluble material, which allows support structures to remove easily after the print. An easier alternative is that snap-away scaffolds are created through a single nozzle 3D printer. These are removed manually once the printer is cooled down to room temperature. In order to produce the complex geometries that are required for medical models then support/scaffold structures are a requirement in order to maintain resolution of the structure. These structures are best deposited using PVA or HIPS material so that they can be devolved through to use of hot water (PVA) or a solvent (HIPS) to leave a smooth finish on the surface of the structure.

The key factor of FDM 3D printing is that the technology is accurate, low cost, and reliable. Machines also have a relatively small footprint size so they can be used within an office. There is also no need for time consuming post-processing required as the models, tool, and devices produced are already finished to a good standard.

During the FDM 3D printing process, the firmament is extruded through a 100–400 μm diameter nozzle. This thin thread cools quickly after deposition to a solid layer structure. The thermoplastic filament is drawn from a reel via the extruders main drive gear. This is used to supply material to the hot-end. The nozzle head heats the material and the drive gear turns to turn the flow of material on and off. Stepper motors are used to rotate the drive gear and adjust deposition driving the 3D printing process. The head is moved in both horizontal *X*-axis and *Y*-axis, while the build platform moves down (*z*-axis) during the building process.

A heated nozzle deposits molten polymer onto a supportive structure layer-by-layer. Various polymers are used, including acrylonitrile butadiene styrene (ABS), polycarbonate (PC), poly-lactic acid (PLA), high density polyethylene (HDPE), PC/ABS, poly-phenyl-sulfone (PPSU), and high impact polystyrene (HIPS). As shown in Fig. 3.28 parts can be produced with hollow complex structures that can be filled with other polymers or drugs. FDM processes can be restricted in the variation of shapes that may be fabricated. However, using a removable support structure then any form can be fabricated. These thin supports are automatically added to model during processing and are broken away during finishing process.

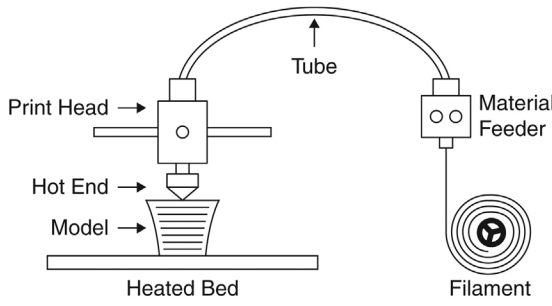

Figure 3.28 FDM 3D printed PCL bone scaffold produced with an internal hollow structure. The structures are used to test the formulation of new polymer blends.

3.7 Stereo lithography

Stereo lithography (SL) was the world's first applied 3D printing process. The process works by selectively curing layers of photosensitive resin. This is achieved through the direction of a laser light that is focused into precise points and cures the resin into a solid structure. SL 3D printing process is highly precise and can be used to form highly complex geometries. The directed laser beam is vectored in the *X*–*Y* axes over the top surface of the resin, and the focused beam cures the resin at the focal point. As soon as a layer cures, the build platform within the tray lowers in the *Z* axis and the next layer is produced. This sequential process continues until the medical model or healthcare component is completed (Fig. 3.29).

The precise and intricate nature of the SL process means that it requires support structures for the majority of the parts produced. This is the case where structures need to have overhangs and undercuts. These scaffolds are removed manually after the 3D printing process. Subsequently, parts are cleaned using isopropanol and cured in a UV curing chamber to finally harden the resin. SL is currently the most accurate 3D printing process which produces an excellent surface finish. However, for applications in healthcare the necessity for post-processing is a limitation. Over time the material can become more brittle and models need to be kept out of direct sunlight.

Digital light processing (DLP) is a similar process to stereo lithography; in that it also uses the light reactive photopolymers. DLP has become common because it uses a conventional light source, and these are often liquid crystal-based, which is applied to the whole layer at a time during a single pass, making it significantly faster than SL.

The process converts liquid polymer resins and composites into solid layers using photo curing UV blue light. A shallow vat of photosensitive resin is exposed to controlled light, as the layer solidifies; then the build platform moves up and liquid resin is again exposed to light. The process repeats until the model has been built.

This process generally costs higher due to the current high price of the photosensitive resins. For the purpose of highly detailed models, the product is of a processional standard. DLP technology is currently the paramount 3D printing technology, which produces 10–25 μm layers to a high degree of precision. This technology can be used to produce high resolution and high-quality parts for biomedical applications or

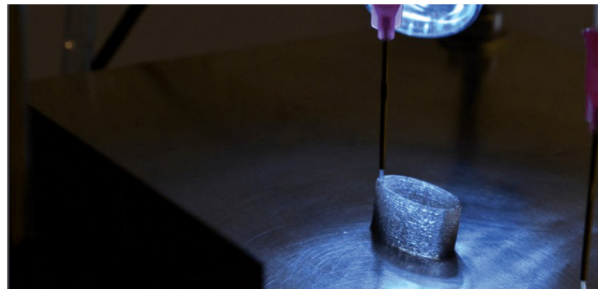

Figure 3.29 Schematic of the SL 3D printing process showing the formation of the part inside a liquid resin tray.

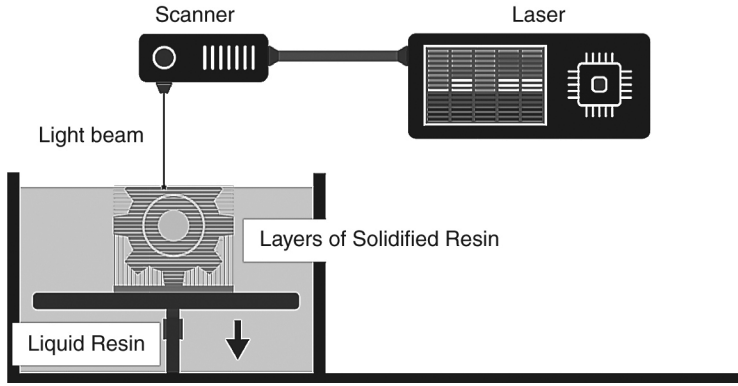

Figure 3.30 A typical DLP 3D printer (left) that is used to produce 3D printed plastic surgery models (right) to a high degree of accuracy.

components with integrated moving parts. Fig. 3.30 shows a typical standalone DLP printer and the model that has been produced.

The process uses mask-image-projection, in which a 3D digital model is sliced by a set of horizontal sections. Each slice is converted into a two-dimensional mask image, and it is this mask image that is projected onto a photocurable liquid resin surface. This 3D printing technique has been used to create highly complex 3D models and structures, and the rate at which models can be produced is impressive. Recent research has resulted in the process being developed so that it can produce components in minutes. It is as a result a perfect complementary technology for dental and plastic surgery [28].

Micro-sized features can also be produced using this technology through the process of multi-photon photo-polymerization. This approach uses a focused laser to trace the desired 3D object [30]. It is due to the nonlinear nature of photo excitation, the polymer is cured to a solid only in the places where the laser was focused while the remaining material is then washed away. Feature sizes of 100 nm are capable of being produced, as well as complex biological structures [31].

The process of DLP 3D printing is shown in Fig. 3.31 and produced highly accurate parts with excellent resolution. A key advantage of DLP 3D printing is that the process sues far less resin in comparison with SL technology. It is less wasteful and is comparatively less expensive.

3.8 Selective laser sintering

Selective Laser sintering (SLS) works on the process of laser fusing power-based materials that include alloys, polymers, hybrid materials, and ceramics. A high-power laser is focused onto a precise *X–Y* axis position on a tightly compacted layer of powder-based material. When the laser energy interacts with the surface of the powdered material, it sinters the particles together to form a solid object. As each layer

Figure 3.31 Schematic representation of the DLP 3D printing process that is used to produce highly complex models quickly and accurately.

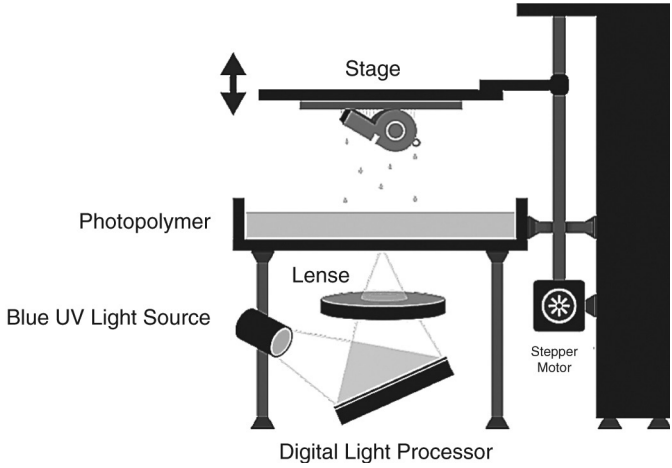

Figure 3.32 Schematic of the SLS 3D printing process showing the production of a 3D part.

is processed, the powder bed drops incrementally and a roller spreads a new layer of powder over the surface of the bed prior to the next pass of the laser. Subsequently, new layers are formed and fused with the previous layer (Fig. 3.32).

The enclosed build chamber is sealed to ensure that the layer of powder material is not disturbed during the 3D printing process. Once the process is finished, the 3D printed model is removed from the machine and the excess powder is removed to leave the final product. A specific advantage of SLS is that that the powder bed serves as a support structure for overhangs and undercuts. Because of this, complex geometries can be made. Porosity and temperature have historically been issues with this process, so the use of this technology for creating structural parts such as that one is shown in Fig. 3.33 is difficult.

The SLS process employs a method of joining and binding differs for the above systems, in that it employs a laser while other methods can use hybrid mechanisms to

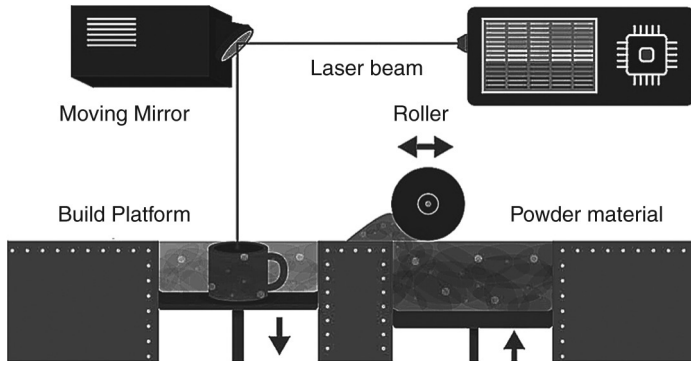

Figure 3.33 Examples of final SLS hip component produced from titanium powders.

join materials together. By putting different powders or particles materials into an SLS process then 3D components can be produced with desirable structural and aesthetic properties. It was this research which allows for more realistic objects to be 3D printed with different contrasts and texture.

3.9 Inkjet binder jetting

The 3D inkjet processes prints one layer at a time. A liquid binder is used in place of an ink to bond together a powder. The build platform drops one layer at a time so that a new layer of powder is spread, to which the machine prints the next layer. This process repeats, layer-by-layer, until the model is complete. This process is capable of making multicolored and complex moving parts.

An inkjet printer head systems are used to spray photopolymer materials onto a build tray in ultra-thin layers between 10 and 30 µm thick. Each photopolymer layer is cured with UV light after it is jetted, producing fully cured models that can be handled and used immediately, without post-curing. A PVA-based support material, which is designed to support complicated geometries, is removed by adding the model to water after the printing process.

Binder jetting 3D printing is where the material being jetted is a binder and is selectively sprayed into a powder bed of the part material to fuse it a layer at a time to create a required part. As is the case with other powder bed systems, once a layer is completed, the powder bed drops incrementally and a roller or blade smoothens the powder over the surface of the bed, prior to the next pass of the jet heads, with the binder for the subsequent layer to be formed and fused with the previous layer. The process is shown in Fig. 3.34.

Advantages of this process, like with SLS, include the fact that the need for supports is not required because the powder bed itself provides this functionality. Furthermore, a range of different materials can be used, including ceramics and biomaterials. A further distinct advantage of the process is the ability to easily add a full

Figure 3.34 Schematic of the inkjet binder jetting process.

color palette which can be added to the binder. The parts resulting directly from the machine, however, are not as strong as with the sintering process and require post-processing to ensure durability.

3.10 3D-bioprinting

Out of all the current 3D printing technologies in medicine and healthcare, none are so powerful as that of the application of 3D-bioprinting. This is a complex domain, combining materials science, electronics, mechanical and software engineering, as well as cell biology and stem-cell science. However, as a technology, it is understandably more complex than any of the other conventional 3D printing methods. The importance of 3D-bioprinting is that one day it could revolutionize healthcare, improve the efficiency, in which replacement tissues are created, and subsequently improve the lives of millions of people.

The basis of 3D bioprinting is that it is an extrusion technology that enables the deposition of combined hydrogel scaffolds and many different biologically active construct types. These can be differentiated, using the deposition of different protein growth factors to transform the tissue into specific vascularized and innervated layers. In order to bioprint living tissues, it is necessary to develop the technology for scalable, accurate, and repeatable deposition these living materials. However, the basis of being able to create complex tissues can only be achieved if we are able to use the natural properties of living cells to self-differentiate. This can be engineered by employing computer numerically controlled automation technologies. As a result, this will need four key enabling concepts in order to build a successful bioprinting system:

1 Dual variable speed-controlled precision syringe pump drivers and a three-axis computer numerical controlled chassis. A syringe pump systems which can be autonomously filled with different cell cultures on demand is the most efficient method for deposition.

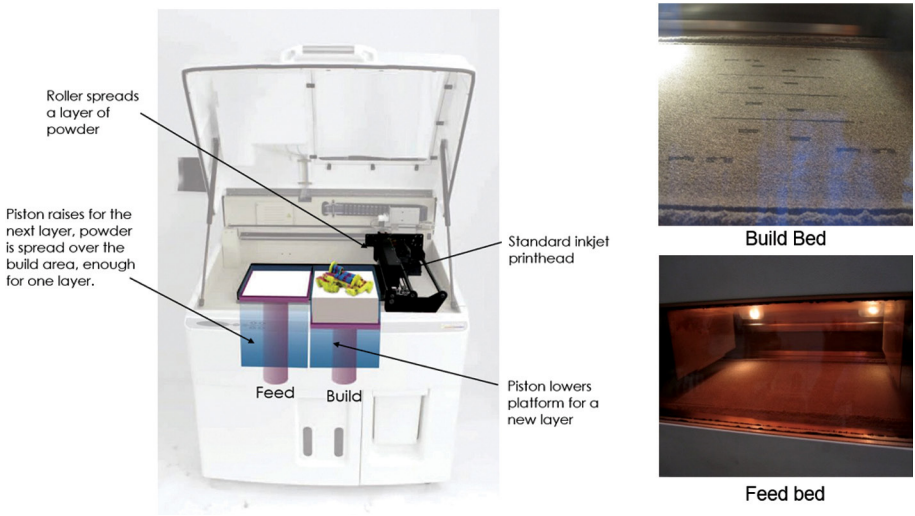

Figure 3.35 The authors prototype 3D bioprinter built for hospital use. This dual extrusion bioprinter was highly accurate and was able to produce tissues which had measurable mechanical, metabolic, and functional properties.

2 Allow for the deposition and living cellular constructs and hydrogel scaffold and/or IR curable material scaffold to produce a self-contained construct.
3 Differentiate cells in layers by applying different protein growth factors.
4 Develop a software tool to control the deposition and fabrication of multilayer complex tissues.

By merging these foundation concepts together, this process allows for the accurate control and deposition of cell-based materials and scaffolds within a 3D space to build complex tissue architectures [32]. Fig. 3.35 shows the Authors experimental 3D bioprinter made for the hospital environment.

A 3D bioprinter is controlled digitally through the use of a 3D control software; the interface is shown in Fig. 3.36 and allows for the control of extrusion speed and temperature.

Over the past 8 years of developing precision 3D bioprinters., it was found that the process is indeed able to fabricate complex biological structures. The step-by-step process used to Bioprint a tissue structure is as follows:

1 A precision syringe driver is loaded with a bio-ink made up of a hydrogel-based (gelatin, agarose, antibiotics, and sucrose) biogel containing 35 million mesenchymal stem cells (MSC) per mL. A second syringe driver containing a special IR photoactive hydrogel/collagen scaffold, which also has added protein growth factors.
2 Software control systems are used to control the bioprinter and instruct each of the high precision stepper motors in 3D space to deposit the different materials in layers. These layers act as an independent support to the 3D structure.
3 The three-axis system needs to position syringe drivers to fill a hollow scaffold structure with cells. The cells are built up layer-by-layer between hydrogel layers until the tissue is

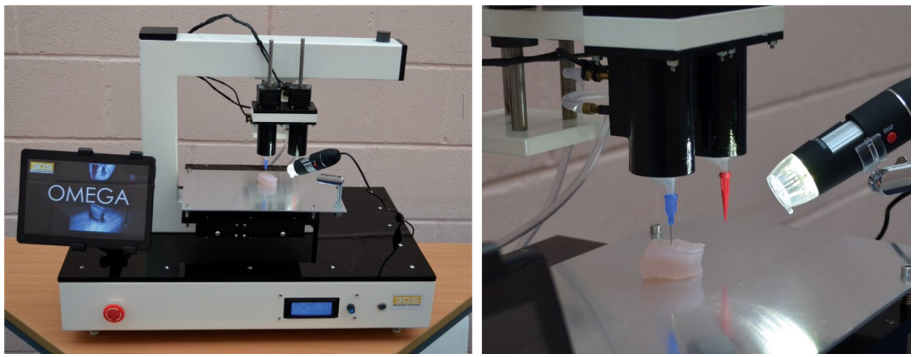

Figure 3.36 User interface of the software control system that is used to accurately position the 3D bioprinter in three axes.

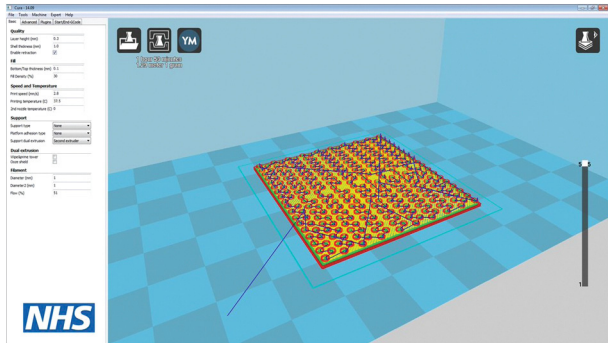

Figure 3.37 3D bioprinted structures produced (left) a knee implant made using Mesenchymal Stem Cells and (right) a small ear structure being cross-linked following the bioprinting process.

built. Photocurable polymers are effective to make artificial vascularized macro-channels for nutrients and oxygen to be applied to the cells.

4 Once the process is finished, the bioprinted tissue is placed into a bioreactor. A bioreactor helps to maintain viability of tissue constructs and gives time necessary for post process tissue maturation, fusion, and remodeling. Bioreactor processing can be used in combination with growth factors that promote angiogenesis and innervation as well as factors that can maintain or preserve cell viability.

5 During a period of time in a bioreactor then micro-environmental parameters such as temperature, pH, nutrient and gas concentrations, as well as regulation of specific mechanical stimulations can be maintained. These parameters will require design and engineering for each specific tissue type.

This 3D bioprinting method has been used to generate heterogeneous tissues such as those shown in Fig. 3.37.

3D bioprinting is potentially a very powerful application 3D printing technology. However, the recent media hype has proven to have hampered the technology and

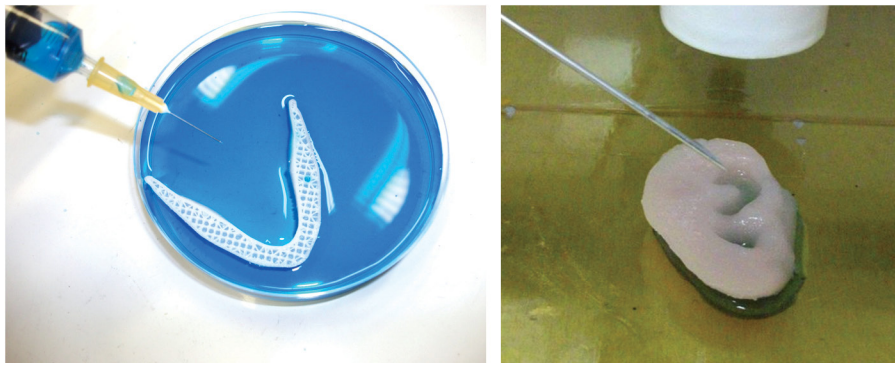

Figure 3.38 3D bioprinting technology used to generate heterogeneous tissues using a printable bioactive gel. Using a process which is driven by IR laser curing of a photo-curable scaffold following the deposition of each layer allows for complex layer systems to be produced.

the promise of it being able to generate complex organs is not correct [33]. However, the application of heterogeneous tissues for transplantation is highly likely in the interim.

In the longer-term 3D bioprinting offers the potential to fabricate organized tissue constructs to replace damaged or diseased human tissues as shown in Fig. 3.38. This directly has a bearing for developing safer and more effective healthcare treatments [34]. This is as such a top down method for engineering new ways toward making treatments more accessible to patients. It also opens up the opportunity for cost effective patient-specific medicine to evolve. The potential to produce functional tissues on demand, made in a controlled and safe way for use in humans may one day revolutionize healthcare and have a huge global impact on healthcare and the economy.

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Prosthetic devices

4

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Chapter outline

4.1 Introduction

Modern prosthetic devices provide enhanced quality of life and restoration of mobility for patients who have suffered the loss of tissue or limbs. Many patients are fitted to a prosthetic device using padding, special manufactured fittings, or minor design modifications during or shortly after the fitting process. In some patients, due to the overall complex nature of the condition, then current prosthetic devices can be insufficient. As a result of the development of 3D printing technology, then it provides a better solution for patients. As a result, there is the possibility for the creation of custom interface between each patient and an artificial limb or synthetic tissue substitute. This can result in perfect fitting that can enhance mobility and provide an improved user experience.

4.2 Medical applications of 3D printing in prosthetic devices

As 3D printing methods have improved and newer advanced materials have been introduced, then the ability to create a range of 3D printed prosthetic devices has developed. 3D printing has allowed for the ability to produce prosthetic devices that are customized to the needs of a patient. These are manufactured to a high degree of accuracy and these are structurally durable and parts can be 3D printed from carbon fiber with fine features and smooth surface finishes produced.

As a result, 3D printing offers a way toward the ultraprecise patient-specific prosthetic devices to be generated at the point of care. Because prosthetic devices can be cost effectively made to be patient specific, then they can in many circumstances

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improve patient recovery time. Fused deposition modeling (FDM) 3D printed parts are now commonly used in a range of orthopedic, oncology, plastics, and pediatric prosthetics.

Because of the integrated software tools such as Blender, then designers can rapidly produce precision 3D printed prosthetic devices. As a result, newer prosthetic devices can also be generated that go beyond the current state of the art. This allows designers to quickly prototype devices, fixings, and tools in-house, from concept models to a fully functional prosthetic device quickly and efficiently. The 3D printed materials are as a result enhanced over that of conventional devices. For instance, 3D printed Nylon 265 has significantly enhanced properties over conventional titanium alloys. Improvements unclosing wear resistance, pliability, and ultrahigh strength, this also comes at a fraction of the price over conventional titanium.

Medical device engineers and designs are as a result able to develop their very own concept prosthetic devices and generate working prototypes in hours rather than weeks. As a result, complex design iterations can be fabricated in a fraction of the time usually required. It is particularly the case that patient-specific limb prosthetics are particularly a paramount example of the benefits of 3D printing in medicine. These have led to a better fit for the patient, better recovery outcomes, and a better experience for patients. These 3D printed devices have been used to help people not just to walk, but to run, swim, and even climb mountains.

4.3 Cosmetic prosthetics

Cosmetic prostheses are a family of interface devices used for aesthetic improvement. They also provide an extension of the limb and can restore tissue, the look of a tissue. As shown in Fig. 4.1A is that of a 3D printed hand prosthetic, these devices come in a variety of shapes, orientations, colors, and sizes. All these elements can be controlled

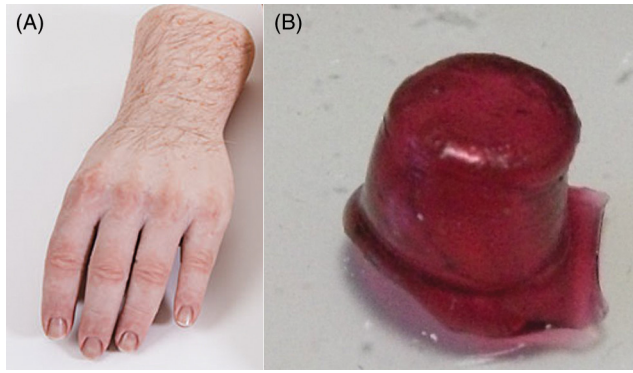

Figure 4.1 (A) Passive hand prosthetic with different designs and materials used to resemble a normal human hand and (B) 3D printed nipple and areola complex.

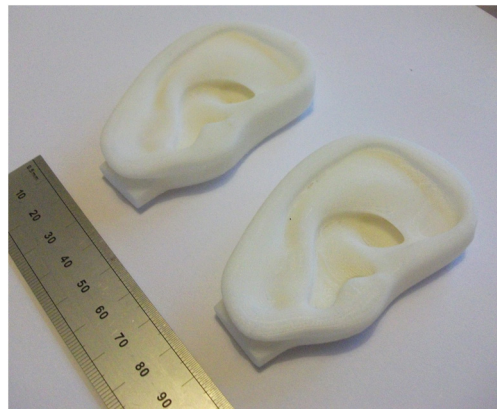

Figure 4.2 Larger complex ear structures 3D printed from medical grade silicone.

via the 3D printing process. The design and polyurethane materials are used to create highly accurate structures.

This is achieved to mimic a hand based upon a database of designs that have been scanned from other patients and can be altered and scaled to suit the needs of a patient. In the case of unilateral amputees, the remaining hand can be 3D laser scanned and this artefact can be mirrored and then 3D printed. The hand as a result perfectly resembles that of the amputated hand.

Imperfections can be designed into the scan to enhance the realism of the design. Further to this, with mechanical structures engineered into the 3D printed hand, then passive prosthetic devices can be used to act as a support or aid the other hand when gripping or holding objects.

Further to this, the nipple and areola complex is another example of a 3D printed cosmetic prosthetic implant. Fig. 4.1B shows the author's fabrication of a prototype structure provided to aid reconstructive breast cancer surgery. This small prosthetic structure had been 3D printed from polydimethylsiloxane (PDMS). This prosthetic device has been designed to be 3D printed and implanted under the skin.

A further example of a larger complex structure that has been produced is that of the ear structure (Fig. 4.2). Here, a medical grade silicone 3D printing filament has been 3D printed using FDM technology. These prosthetic structures can later be painted and attached to a patient. This example of 3D printed has been designed to be cost effective as a method in the first instance of treating microtia.

4.4 Body powered prosthetics

A 3D printed body powered prosthesis uses bodily movement and/or muscle actuation of a patient to mechanically control the movements of a device. This is usually controlled via a body interfacing. With the use of cables and/or pulleys, these are integrated in both the terminal device and the harness. The resulting tension created

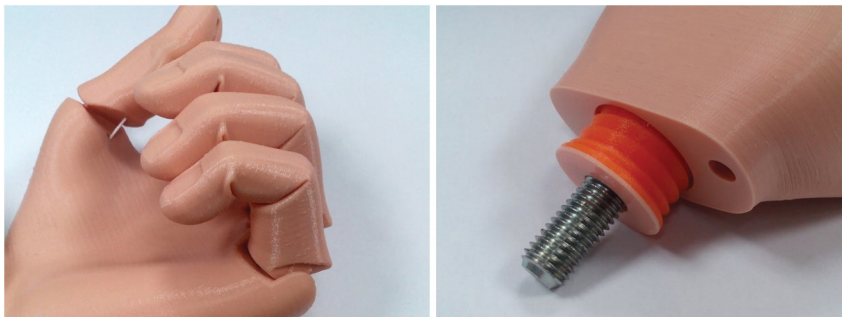

Figure 4.3 A 3D printed body powered prosthetic devices with integrated moving figure structures.

subsequently pull the cables and move the prosthesis. Whereas the previous generation of body powered prostheses used split-hooks or grippers, the modern 3D printed equivalent is formed into the shape of a realistic hand as shown in Fig. 4.3. These amazing devices have increased functionality in the fingers and they also feature threaded connectors for durability when connecting it to a patient.

The devices shown above is kept in preset position, either open or close, by a series of polymer elastics and for movement an opposite force is generated in these bands. It is this method that is used in the many body powered prostheses today, which produce a force in one direction. Due to the mechanics of the design, the device can only be controlled in a single direction with devices divided into voluntary opening and the prosthetic device in a naturally closed position, or voluntary closing, which is the opposite and the device is open in a relaxed position.

Each mode, voluntary closing, or voluntary opening is dependent on the patient's requirements and depends on the required need and function. The benefit of 3D printing is that it gives a patient the opportunity to have both types of prosthetics to be manufactured and used when needed. A key advantage of 3D printed voluntary closing prosthetics is that the grip strength can be adjusted. By using novel 3D printing materials, then the devices have a high degree of durability. Voluntary opening systems offer the opportunity of allowing the patient to relax when holding onto or carrying an object, instead of applying a constant force, which is needed with voluntary closing devices.

4.5 Bionic prosthetics

New prosthetics technology and designs that have great potential is that of bionic prosthetics. These are externally powered with the use of microservos and stepper motors, which are controlled by electrical signals produced in a muscle when contracted. By using electromyography sensors against the muscle region then this register potential changes in activated muscles.

The electromyography sensors are located precisely on the skin near the targeted muscle. By using 3D printing technology, then the precise position of the interface

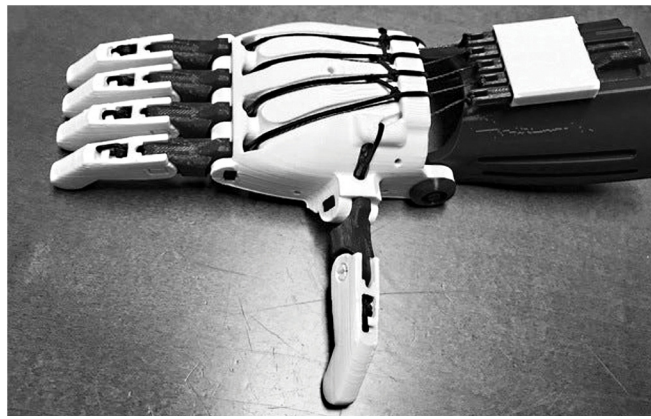

Figure 4.4 A 3D printed bionic hand with integrated finger positioning technology. Here, the device is fully open, however, each figure can be controlled independently.

can be designed into the prosthetic impact accurately. The electrical intentional contraction impulse signal is used as commands for the terminal device. As a result, it causes the bionic device to move in a specific way. 3D printed devices have developed extensively over the past five years and consist of complete electrical and mechanical devices that can achieve a range of different hand movements.

Increased precision and dexterity are now being developed, and with the era of micro-3D printing then more complex integrated devices can be fabricated. One such prototype device is shown in Fig. 4.4. With the integration of reliable power supplies and advanced 3D printing materials then this prosthetics technology will continue to develop.

Further exciting developments in this technology are in which electromyograph sensors are implanted onto the muscle itself. This has a key advantage of ensuring a reliable and accurate signal is received. An unattenuated signal ensures that the control of the device is highly accurate, and the movement of the devices is highly precise.

By using 3D printing technology that the expense of conventional devices can be reduced. Polymer technology also ensures that the device can be built to be lightweight and custom made. These types of prosthetics are growing in demand due to the improvement of signals processing technology and the increased functionality. As a result, multiaxis movement is now possible, and devices, which consist of two joint movements are now possible.

4.6 Prosthetic socket fittings

The interface between the prosthetic device and the human body can be particularly problematic. The socket fitting is the part where the residual limb meets the prosthetic device. Its impact directly affects the comfort and function of the device. It is an important element that can be 3D printed to fit perfectly.

The importance of 3D printing socket structures is that it minimizes shape geometries that can cause significant discomfort. The structure can also be designed to fit the patient specifically and thus minimizing the risk of discomfort as a result of blisters. Because of the complex geometrical shape of the human body then the 3D printing process can be used to create a fit so that the use of the prosthetic matches the needs of the patient. Prosthetic structures can be made so that the structure ensures an even distribution of mechanical load across a wide surface area. As a result, this reduces pressure and maintains a key alignment that results in greater comfort and less wear. This is particularly the case as a young patient's age. Using 3D printing technology, then the design can be updated over the life of the patient. The various methods for creating a connection between the prosthesis and the limp include:

- Suction sockets are perfect if the stump has a smooth surface. Here, a 3D printed one-way valve produces a vacuum inside the socket. The connection is therefore flawless and fits well for patients.
- Self-suspension sockets are designed using Blender and 3D printed to match the limb fitting exactly. This type of fitting is fitted exactly and held precisely in place by the socket gripping around the limb. This is a key advantage of using 3D printing where the device can be 3D printed to fit a complex shape of the limb.
- Harness sockets: A harness can also be used independently, if the suction and self-suspension sockets cannot be used. The harness creates good support and is a dependable option for prosthetic users.

Each socket starts with the 3D laser scan of the stump to create a 3D model. This model is then used to creative a negative socket design so that the patient can be accurately matched up with the structure. Because there are a range of transparent 3D printing materials that can be produced flawlessly, then a perfect vacuum can be produced for the user. It is this perfect fitting, which is important to ensure patient's comfort.

The benefit of using 3D laser scanning is that the digital model can be accurately adjusted before the 3D printing process. The novel method for the creation of fittings has a wide degree of potential and could very well become the most cost-effective method for the creation of implant structures. Also, this will reduce working time of a prosthetist, and the resulting scan is easy to adjust to better fit the user.

4.7 Advanced prosthetic devices

The next-generation of 3D printed prosthetic devices will be fully functional structures that allow for a wide range of movements and multiaxis freedom. With 3D printing technology, then multiple joints and structures can be fabricated to move and rotate to their required positions. This is particularly the case with finger structures and intricate parts that need to move individually. These advanced prosthetic devices will be able to grip complex structures and move independently of each other. Fig. 4.5 shows a range of current 3D printed bodily parts that can be integrated together to form a range of next-generation prosthetic devices.

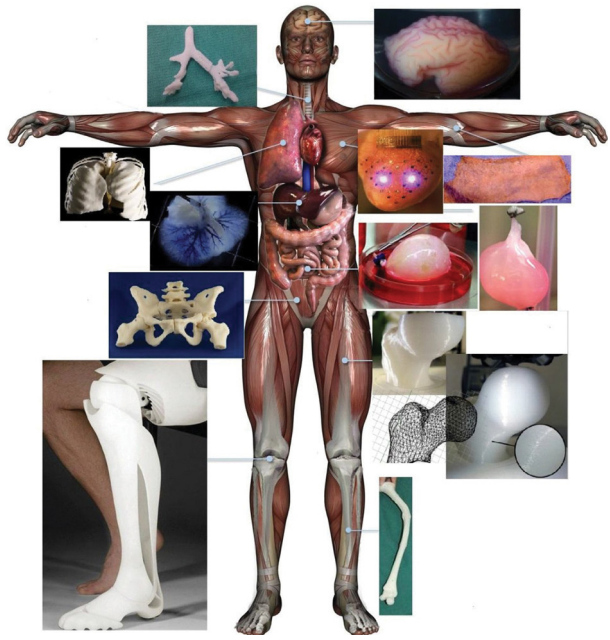

Figure 4.5 Example of the wide range of 3D printed bodily structures that have been fabricated.

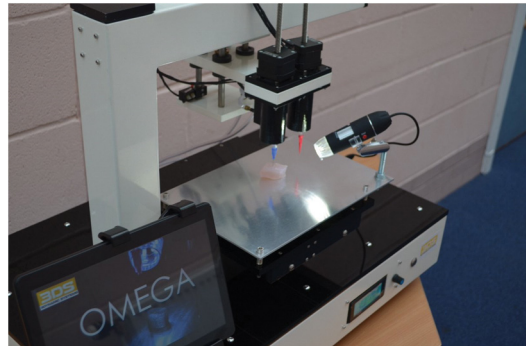

Figure 4.6 The fabrication of a prosthetic dermal filler that can be produced to perfectly match the shape of the patient on the authors prototype 3D printer built to allow for the fabrication of a range of advanced materials.

The importance of 3D printing in the fabrication of bodily parts is that a range of advanced geometries can be fabricated out of advanced high strength materials. These can be made from carbon fiber, high strength nylon, or flexible polymers such as PDMS or polyurethane. Fig. 4.6 shows the fabrication of prosthetic dermal filler that can be produced to perfectly match the shape of the patient.

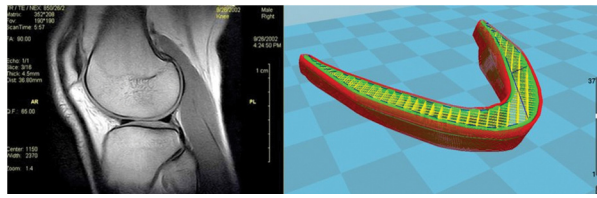

Figure 4.7 Rebuild of an integrated meniscus disk implanted prosthetic device.

The key reason why this technology can take shape is due to the fact that 3D printing requires very little skills to manufacture any complex part. Since the technology has reduced the cost significantly over recent years, it is becoming more accessible.

It is the development and accessibility of the 3D printing technology that has accelerated the harnessing of 3D printed prostheses. With the requirement for the fabrication of advanced prosthetics, then the possibility to create accessible patient-specific medicine will evolve.

Fig. 4.7 shows the current state of the art in the fabrication of integrated implanted prosthetic devices. These are designed to integrate with the body and for the integration of tissue systems. This is particularly the case for producing prosthetics for children who will quickly grow out of a device.

Facial prosthetic implants as shown in Fig. 4.8 are also another avenue that opens up the direct 3D printing of complex geometrical structures. The structures can as a result be fabricated accurately to a high degree of precision. These new technologies are fully integrated and are now being used by a range of medical practitioners.

Direct 3D printing of PDMS polymer with the correct color and texture matched to the patient is shown in Fig. 4.9. This part needs very little finishing and can be worn comfortably by the patient. The rebuild of this structure was carried out by scanning the patients opposite ear.

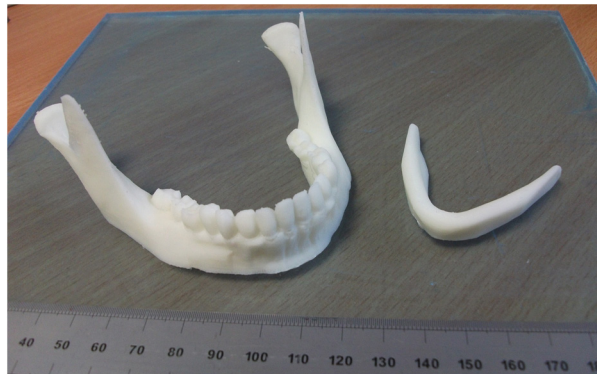

Figure 4.8 Two 3D printed dental prosthetic structures produced from CT scans of a jaw features.

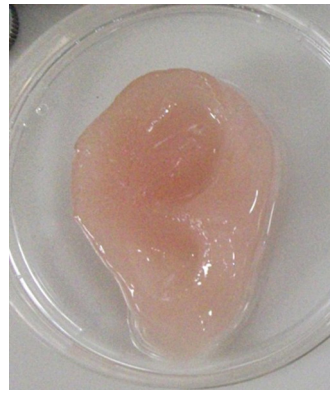

Figure 4.9 A photography of a natural ear prosthetic device 3D printed from PDMS with the correct color and texture match.

4.8 Conclusion

In this chapter, we have demonstrated many key advantages of using a wide range of 3D printed prosthetic devices. For this purpose, 3D printing technology is the paramount method for the creation of the complex geometries that are patient specific. It leads toward the ability for healthcare professionals to engineer devices specifically for each patient and in a cost-effective manner. It has been seen that together with 3D laser scanning and CT scanning technology, complex geometries can be produced. Due to the vast array of advanced materials that are available for 3D printing, then mechanical durability requirements for such parts can be met. It is the healthcare requirements for prosthetic devices to be made patient centric that allows 3D printing to produce fully functional devices.

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Operative models

5

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Chapter outline

5.1 Introduction

Recently, 3D printing has been introduced as one of the most effective and successful technologies in the medical fields, enabling anatomy comprehension through the distinctive features of the 3D printed models [1–4]. These features include high accuracy and customization based on patient's scan data and such models are solid and graspable and can be manipulated by the user for better understanding of the anatomical details, particularly the augmented sensory perception [1,3]. This feedback combined with visual inputs lead to accurate observed and perceived details. Retrieving information from 3D printed models is faster, easier, and time-safer, when compared to 2D/3D imaging assessment [5–7].

Uses of 3D models in medicine include counselling with patients, medical education and training, preoperative planning, intraoperative navigation and simulation of surgical tasks, and customization in the form of patient specific implants and/or prostheses [1,3,8]. The present chapter illustrates some of the innovative uses of 3D printed operative models in treating surgical cases.

5.2 Virtual preoperative surgical rehearsal

Various uses of the 3D printed anatomic models have attracted the attention of researchers, medical students, residents, and fellows. These uses included the virtual surgical planning and/or surgical simulation, and other procedural training [1, 3]. Preparing the patient for a surgery necessitates a full and a clear understanding of the target anatomy. This can occur only through the preoperative setting which provides the surgeon with a plan to the targeted operation.

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The interpretation of the available images is considered the first step that would help any surgeon. Traditionally, these images or pictures are 2D derived from conventional radiology. Once they are analyzed, it becomes surgeons' responsibility to mentally reconstruct the observed flat monitors into 3D architecture. And it all depends on surgeon's ability and experience to optimize treatment option during surgery [9]. However, preoperative 3D planning and anatomical models allow various surgical treatment options without causing any harm to the patient or exert extra loads on surgeon's ability. The 3D anatomical models are built into a solid volume, where the user can observe and manipulate them, followed by the visual and tactile feedbacks [10,11]. Thus, tracing and revisiting of surgery can be done through the model, which allows anticipation of any technical challenges due to undesirable anatomy or to disease-related alterations [12]. Thus, knowing and understanding patient's anatomy before the surgery is considered a great achievement [10,11] as it has proved its effectiveness in presurgical decision making of different specialists (e.g., brain, cardiovascular, maxillofacial, transplant and general surgery, and orthopedics) [3,12].

For example, 3D planning can be integral in tracking nerve pathway in surgery performed adjacent to crucial nerves as previously reported [13]. Preoperative mapping of mandibular nerve is a must prior to surgical intervention to excise excess bone of mandible body as in treating mandibular hyperplasia (Fig. 5.1).

Furthermore, 3D printing assisted in deciding at-risk structures through drawing resection lines or dissection planes, which in turn, assess the spread of disease to be ablated. Some studies have confirmed the retrieved level of accuracy by the extremely low margin of error in terms of mm when users were asked to estimate measures (e.g., distances, lengths, or volumes) both on 3D printed models and other image platforms such as MDCT or 3DV [5,7,8,11,14–19]. Also, some studies reported saving 30 min

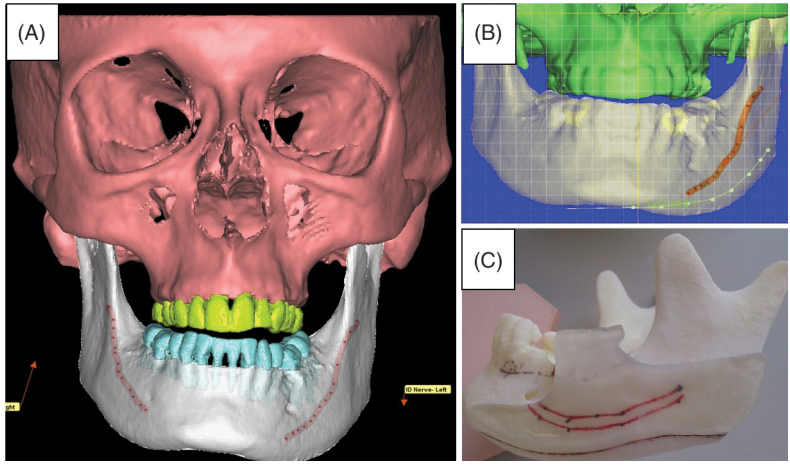

Figure 5.1 Mapping left side mandibular nerve (A) proved crucial in planning the excision of excess bone on left side as defined by the cutting line in relation to the nerve (B). It enabled the design of cutting guide that maintained the nerve (C).

of operative time when pre-procedure 3D models were used in pediatric cerebral vascular malformations surgeries [20–22].

On the other hand, although 3D virtual planning has facilitated the process of understanding anatomy, the complex process of such operations become more comprehensible with experience. Junior surgeons find it difficult to deal with the limited availability of the needed specialized software in 3D virtual planning [5,23]. Thus, experience factor is a decisive one when anatomy is more irregular due variations or disease-related distortions [5,23,24].

5.3 Treatment of fracture

The use of 3D-printed models for operative planning has been implemented mainly to plan internal fixation and plating in many medical disciplines. These models play an important role in the preoperative planning stage, as they assist in determining the appropriate method of fracture reduction. Surgeons, in some cases, are also able to sterilize the models and use them intraoperatively. In severe cases where complex fracture occurs, 3D modeling allows surgeons to preoperatively mold malleable plates to the configuration of the fracture. Consequently, operative time and costs would be reduced, and definitely better outcomes would result (Fig. 5.2) [25,26].

In some cases, virtual planning in fracture treatment involves segmenting and mirror imaging of nondefect side to produce a 3D model that is used in customizing an implant that is used in restoring missing bones or contours (Fig. 5.3).

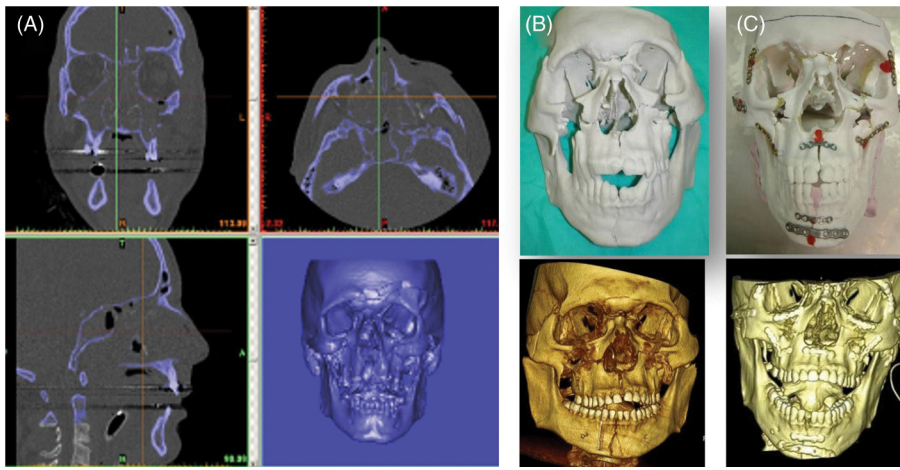

Figure 5.2 Pan-facial trauma resulting in comminuted bone fractures as shown via the virtual reconstruction of patient scan (A). 3D model was produced to visualize and diagnose fracture lines (B). Fractured bone reassembled in ideal locations and fixing plates were bent accordingly, which were used in theater (C) (acknowledgment: Mr Phil Hollows; Queens Medical Centre, Nottingham, UK).

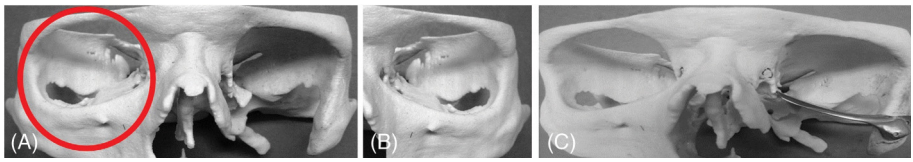

Figure 5.3 Left orbital fracture shown on 3D model (A) where a mirror image of the right orbit (circled) was 3D printed (B) and used in manipulating a plate that restores missing left contours to exact symmetry with the right orbit (C).

5.4 Resection of tumors

3D printing of anatomic models for preoperative planning has been used in planning the surgical approach for resection of tumors in different medical specialties. Clear example would be "2 in 1" meningioma resection surgeries (Fig. 5.4). In such surgeries, it is crucial to define amount of bone to be removed, anticipate resultant defect and its effects, and size and volume of implant needed to cover the defect. Thus, virtual surgery is usually performed and 3D model is produced to facilitate construction of cutting guide that follows virtually planned cut margins, and allows design and fabrication of reconstructive implant that is fixed in place restoring bone removed thus reinstating aesthetics and function [27].

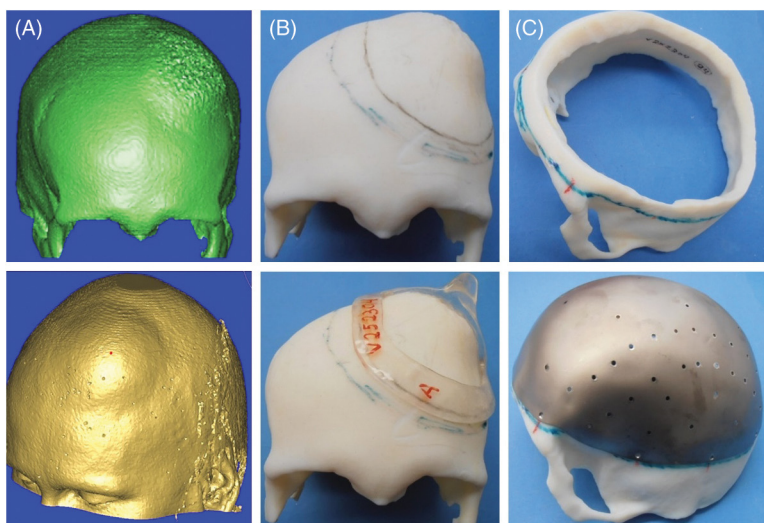

Figure 5.4 Meningioma case where bone tumor is showing through the parietal and frontal bones on the skull left side (A). 3D model and cutting guide of meningioma resection were produced (B). The tumor was virtually resected and 3D model produced onto which an implant was fabricated (C).

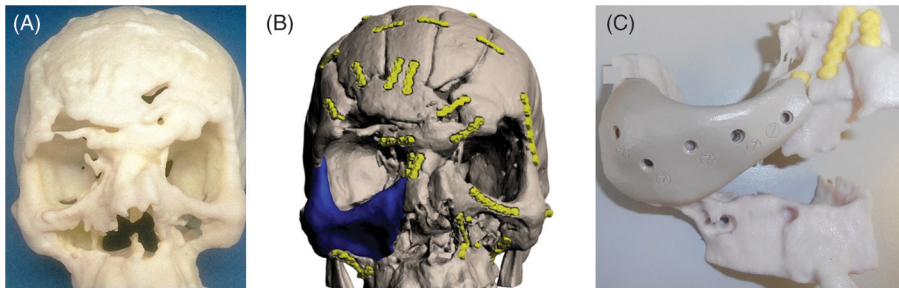

Figure 5.5 Example of pan-facial trauma case where patient is shown a 3D model of his diagnosis (missing bone including left orbital rim and zygoma) (A); the planned virtual reconstruction (B) and 3D milled PEEK implant to restore the defect bone (C).

5.5 Patient engagement and consenting

Preoperative models positively help to engage patients in decisions related to planned treatment. Having a 3D model of abnormal pathology to be treated aids medical practitioner to explain to patient their medical condition in lay language, demonstrate surgery to be performed and outcome to be achieved, hence improving patient's understanding of their disease and treatment to be performed. Also, in complex abnormal pathology, replicas showing the pathology, which patients can visualize and manipulate are thought to help understanding the relative anatomical structures, the abnormal pathology and the necessary intervention (i.e., surgery) [28]. Furthermore, in cases where a custom-made implant to be inserted; patient will understand better the implant, visualize it and have a tactical sensory perception of it prior to sterilization. Such experience will increase patient's confidence and help their consent (Fig. 5.5).

5.6 Conclusion

3D printing is one of the most innovative technologies that has revolutionized the medical practice, especially preoperative planning and modeling. Earlier cases emphasized the need for surgery planning and customization for virtual operative rehearsal, fracture fixation, and resection of bone tumor. Such uses can be extended to the various medical specialties. Outcomes were improved in terms of increasing surgeon's confidence through virtual practice of planned surgery; reducing operative time; minimizing postoperative complications; and optimizing treatment outcomes of both cosmetics and function. Current practice of medicine makes the most of this innovative technology as it became affordable, accessible, and personalized. Added to that, the continuous development of the printers increased the level of controlling safety while printing biomaterials.

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Surgical instruments and medical implants

6

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Chapter outline

6.1 Introduction

The wide range of 3D printing applications in medicine has increased extensively over the past 15 years. Over recent years, this has been expanded for the applications of the manufacturing of 3D printed surgical instruments and medical implants. Due to the increased accessibility of 3D printing equipment, then there are good opportunities for the fabrication and utilization of numerous conventional and specialist surgical instruments manufactured at the point of care. This is particularly the case in the developing world where there is an increased demand for the fabrication of instruments. Further enhanced applications that include the fabrication of medical implants for a range of patients.

6.2 3D printed instruments

Since the 1980s, 3D-printed models for surgical planning have been used. This early inception in the technology has then been extended toward the fabrication of clinical training models and educational demonstrators. This interest in 3D printing technology has grown due to the numerous benefits of using 3D printing from the context of surgical; planning, practicing, and optimizing [1].

Following the improvements made from the fabrication of patient-specific anatomical models, then the next step was in the fabrication of surgical instruments. The process required to manufacture precise, cost-effective and custom-made surgical instruments has numerous challenges. A key issue is in ensuring that the components can be sterilized after 3D printing. However, this can be achieved using vaporized hydrogen peroxide, which is perfect for medical grade plastic instruments [2].

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Figure 6.1 3D printed prototype dental surgical instrument that is 3D printed from HIPS.

Due to the inert nature of FDM 3D printed materials, then due to the 220–230°C) deposition temperature, this should in theory sterilize such instruments and parts. However, it is advisable to autoclave all instruments to ensure that they are completely sterile. Fig. 6.1 shows a prototype dental surgical instrument 3D printed from HIPS. This is the example of a structure that can be 3D printed before an operating procedure and potentially used.

There are a number of 3D Printing polymers that have an excellent result when sterilize, whether this be chemical, steam or gamma radiation sterilization, particularly:

- Polyetheretherketone
- Polyimide
- Polycarbonate

Over recent years, 3D printing of surgical instruments is a key benefit for developing countries. The added advantage of creating instruments that are customized to the requirements of the surgeon, then we have a panacea to the development of instruments for new and pioneering surgery.

For a range of surgical disciplines, then there is the potential in fabricating a range of instruments that accounts for the patient size and nature of a procedure. 3D printing also has the advantage that precise and fine-featured parts can be fabricated that may be required for special surgical cases. If there is a requirement for the fabrication of specialist instruments, then 3D printing offers the more paramount method toward treating patients.

It is the aspect of 3D printing surgical instruments for procedures that require scalability that is of interest. This is particularly the case with procedures involving pediatric surgery. It is due to the flexible nature of 3D printing that complex parts and complex geometries can be fabricated. With the integration of dual extrusion 3D printing, then there is the ability to produce advanced surgical instruments made from two

different materials. Hybrid instruments can be produced with a flawless surface finish and require no further finishing.

Because of the requirement for the vast inventory of surgical tools in a modern operating theatre, then this is often expensive to maintain. With limits in storage in some hospitals, then 3D printing offers benefits of producing instruments and components when required. Just in time 3D printing of surgical instruments, tools and or implants can extend the current capabilities of a medical center. Due to the rapid production of parts and components, then 3D printing can fulfill the requirements for the production of any required part. Both selective laser sintering (SLS) and fused deposition modeling (FDM) 3D printing offers a cost-effective way in producing components on demand in a time centric fashion.

It is the developments in CT scanning that has allowed for the production of precise and accurate structures that are perfect to match to the patient requirements. This is particularly the case with orthopedic implants, fixings, and tools that can be designed and fabricated to match the needs of the patient and surgeon as shown in Fig. 6.2.

Although it is the case that the vast majority of surgical instruments will work with the majority of patients, when a patient has unique bodily features or where there is the requirement for complex medical procedures that the true benefit of 3D printing can be realized. There is a significant benefit of the fabrication of tools that allow for an increased control operative experience, which can result in reducing the risk of complications.

Due to the small size of 3D printing equipment, then it is highly advantageous to 3D print parts at the hospital in close proximity to the operating theatre. It has been shown in many instances that 3D printed components can be effectively serialized as can eb used in procedures that require mechanical stability during a procedure. This is particularly the case in the fabrication of surgical drilling and cutting guides.

Further applications in the region of creating specialist and novel surgical instruments have been demonstrated. Over recent years, the mechanical performance of 3D printed surgical instruments has been proven in many cases.

Figure 6.2 A series of 3D printed orthopedic fixings produced to suit the size required. Each is FDM 3D printed from carbon fiber reinforced Nylon 645.

Surgical instrument made from polymers need to have advantages over traditional stainless-steel instruments. The parts must as a result be designed to be durable, lightweight, and adaptive. The mechanical integrity must as a result be maintained during the life of the instrument. Many design iterations can also be produced for the instrument so as to achieve optimization.

As a result, surgical instruments that can be reinforced with materials such as carbon fibers offer superior strength, which pound for pound is in excess of traditional parts. Carbon fiber reinforced nylon for example can be used for the bespoke manufacture of clamps, surgical guides, and fixings. As a result, 3D printed parts that require a high level of mechanical stress induced onto a part. As a result, 3D printed parts offer an optimum solution.

6.3 Surgical planning and training

The ability to 3D print custom-made instruments and medical models offer great value to medical practitioners. This is particularly the case with allowing surgeons further preparation and for communication with patients and other medical practitioners. The ability to create physical models is a real advantage to pioneering surgical procedures. These are particularly the case in which surgeons are assisted with:

- *Planning:* The fabrication of a physical models of an anatomical model or tumor structure allows for the opportunity of improving surgical planning. It is only when being able to visualize a geometry and the possibility on determining a new solution that cannot be revealed by a 2D image alone.
- *Practicing*: By 3D printing models**,** simulated structures, and medical components, then this enhanced a surgeon's ability to practice. This is particularly the case with procedures that involve one or more specialties. As a result, with complicated procedures, then we can better prepare for a procedure or demonstrate risks and difficulties. This can as a result provide an increase in the efficiency of procedures and the potential for enhanced clinical results.
- *Optimizing*: There is the future opportunity for the ability of the complete fit up and practice of a procedure. Here, the patient specific model, surgical instruments, and implantable components are optimized and 3D printed. This will let the surgeon practice a whole procedure beforehand. It is through the production of the complete set of components that there is the ability of the surgical team to further plan, optimize, and change the approach required. As a result, this makes it more suitable to account for any issues in the patients' anatomy.

Fig. 6.3 shows the process in which a 3D medical model is being fabricated. The structure here is being fabricated from a polyurethane material to form a soft structure. The structure is produced layer-by-layer with a curing time of 30 s between the formation of each layer. Many models can be produced one after the other, allowing for the fabrication of models that account for different surgical scenarios [3,4].

3D printing is currently used widely in the manufacture of oral-maxillofacial surgery components. The applications here include surgical planning models and the production of both alloy and polymer implants.

It is becoming increasingly possible for surgeons to have a surgical model produced for the process of planning a procedure. This facilitates the selection of tools

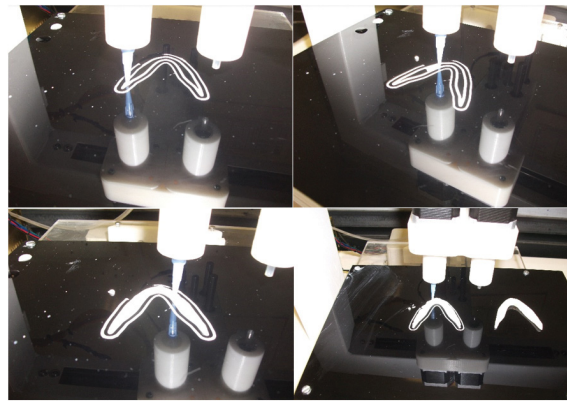

Figure 6.3 The fabrication of medical models made from polyurethane polymer. The model being produced is that of an upper palate.

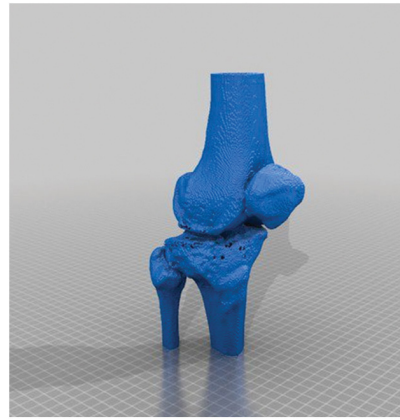

Figure 6.4 3D rebuild of a patient's knee via a CT scan that has been rebuilt to form a 3D structure ready for 3D printing.

and how a procedure is approached. In 2020, we are now seeing that 3D printing for the purpose of surgical planning is becoming a standard practice in hospitals.

Fig. 6.4 shows the direct rebuild of a patient's knee structure ready for the 3D printing process. This model will allow the surgeon to practice a range of techniques before a procedure. With modern technology, then the process through which a 3D printed model is produced from a series of CT scans is now highly accurate and high resolution [5–7].

These models also provide a surgeon with not just the visual but also the tactile and textural accuracy of a patient's anatomy. Following a chemical sterilization process, these models can also be used intraoperatively. There are numerous examples of the

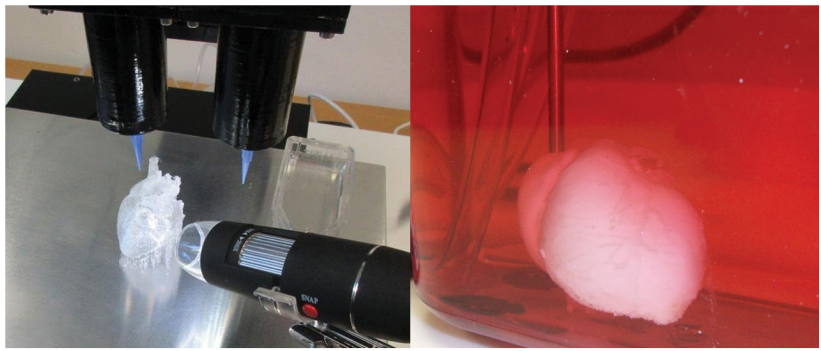

Figure 6.5 (left) The fabrication of an intraoperative model of a young child's heart and (right) chemical sterilization of the structure before use in the operating theatre.

benefits of these models to procedures, including complex pelvic trauma, maxillofacial, complex bone fractures, and osteotomies [8].

It further augments the ability of a surgeon by allowing them to terrain and then to develop their skills for a wide range of procedures [8]. For patients who are about to undergo a complex and potentially life changing surgical procedure, then models also offer the opportunity for communication [9]. Surgeons can as a result kinesthetically explain the procedure to the patient, this can help is breaking down the language barrier. Interactive models as a result bridge the gap in communication between surgeon and patient. For student doctors, it allows for the teaching of both normal and abnormal anatomy. Doing this physically can aid learning and increase visual learning in a 3D environment.

As software tools have improved over recent years, then so has there been a reduction in time and complexity toward the generation of medical models. Fig. 6.5 shows the fabrication and sterilization on a medical heart model. To test the speed at which the model can be fabricated, then structure has been produced for scan, to rebuild and subsequent 3D printing and chemical sterilization in under 6 h. This demonstrates that there is the potential for rapid fabrication of medical models. Here, a transparent polymer has been used to allow for the internal structure of the heart to be visible.

As with anything that is new it takes some time to build up the required infrastructure to allow for a seamless transition of the current to the next generation [9]. As 3D printed patient-specific models become used more in hospitals so does the number of different materials and techniques. Models can also be firstly used for (a) preoperative planning (b) as an aid to explain a procedure to a patient (c) intraoperatively, (d) postoperatively, and then finally (e) used as a teaching aid [10]. There is further scope that models can be held in an inventory for teaching. As a result, there are numerous advantages of 3D printed models:

- It is the difference between patients and the complexities of modern-day medical procedures that makes the use of 3D-printed models a good method for surgical planning.
- This is particularly the case with creating parts that reproduce the size, weight, and texture of a tissue structure or foreign body. As a result, it allows surgeons to plan complicated procedures on 3D models effectively.

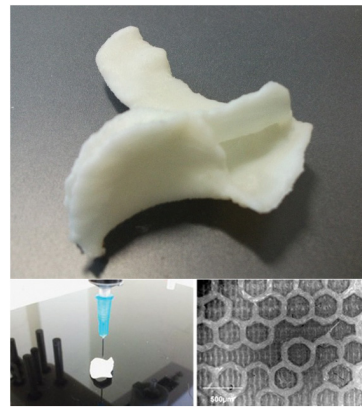

Figure 6.6 3D printing of a facial bone structure and the complex internal trabecular structure produced by the 3D printing process.

- By accurately simulating every stage of a procedure using surgical models can help to overcome surgical complications. These also can result in reduced theatre time and forging a new preoperative planning approach in medicine.

Orthopedic surgery in particular benefits directly from the instigation of 3D printing. Fig. 6.6 shows an example of a 3D printed facial bone. Inside the object is the trabecular detail of the bone structure and the 100% realistic size, shape, and weight of the real structure [11]. The main benefit of using this approach is in the reduction of procedural time and the whole process can be effectively planned. The position of how the surgeon will use the instruments is known before the procedure and as a result the whole process optimized efficiency and time [12].

As a result of the advancement of new materials, then biological structures can be fabricated that have not just the shape and size requirements, but also mimic the texture and mechanical properties. This is shown in Fig. 6.7 in which an ear structure of a small child has been fabricated with identical tactile properties. Tradition display methods for demonstrating such geometries has distinct problems of showing the depth, angle, and texture.

6.4 Point-of-care manufacturing

Point-of-care (POC) fabrication is a novel means to manufacture a wide range of medical implements in a clinical setting. This is particularly the case of the just-in-time fabrication of anatomical models, surgical instruments, prosthetics, scaffolds, and implants at the place of patient care [13,14]. This as a result allows for the evolution in patient-specific medicine where 3D printing is integrated into many different medical procedures. Many hospitals have now embraced such technology to offer onsite fabrication of patient-specific components.

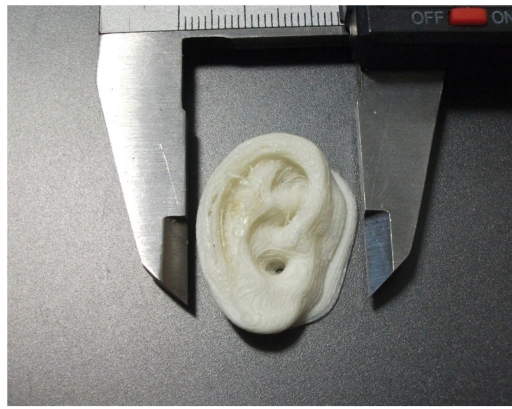

Figure 6.7 Coarse 3D printed structure of a young child's year model. This model has been produced from a latex polymer to mimic the flexibility of the original ear.

Figure 6.8 The fabrication of soft tissue models 3D printed from polyurethane and used for the purpose of surgical planning.

Fig. 6.8 shows two identical 3D printed artery models, which the correct geometrical size, texture, and with the correct mechanical properties as those of the patient. These were fabricated at the POC with the purpose of surgical planning surgery to correct a complex congenital defect.

Due to the acceleration of 3D printing technology, the speed and precision have increased significantly over recent years. Components can be produced accurately out of an increased variety of materials in minutes rather than hours [15]. This is particularly the case with softer materials as shown in Fig. 6.9 in which the structure has been 3D printed before an operating procedure has taken place.

It is the process of desktop 3D printing that is making the process more accessible. It also allows for the final part to be finished within a short period of time. There have been numerous studies that have been carried out regarding the production of complex organ models, prosthetics, instruments, and surgical implant manufacturing at the

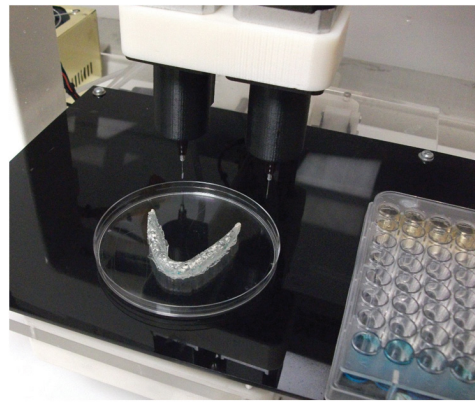

Figure 6.9 3D printed soft structure 3D printed from PDMS polymer. This object was produced in the runup to an operating procedure.

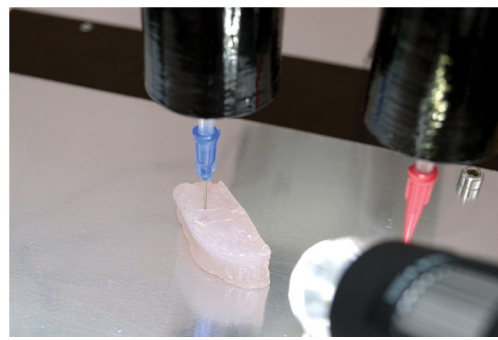

Figure 6.10 Fabrication of the size and geometry of a tumor produced at the POC.

POC. To test the viability of this, we produced the shape and geometry of a 3D printed tumor as shown in Fig. 6.10. It is indeed viable to manufacture surgical models as the process can be achieved quickly with 3D printing. A small tumor model with a size of (20 mm $\times$ 20 mm $\times$ 15 mm) takes approximately half an hour to generate.

Before manufacturing surgical models, it is important to ensure that the correct 3D printable materials are used. They need to be the same texture, color, and stiffness at the real tissue structure. The material used also needs to be sterilizable so that they can potentially be used intraoperatively.

It is effective to produce the structure from CT scans due to the higher resolution and availability of software to convert DICOM2 to a 3D model. We have found that in the context of generating structures that offer an in-depth profile of a biological structure that would be difficult to achieve using conventional digital methods [16]. If a surgeon is provided with a clearer starting point, then this can provide better insight, leading to the potential for better clinical outcomes. This is particularly the case for:

- Complex plastic surgical procedures
- Vascular procedures
- Pancreatic operation planning
- Heart structure and
- Tumor models

The advantages of POC 3D printing is tremendous, particularly as equipment becomes quicker and software tools become easier to use. Matched with the increase in advanced materials available and an increased number of user case studies reinforce the notion that 3D printing can be used in a range of procedures. As equipment reduced in price, then the accessibility increases further to make the possibility that 3D printing has a place in every hospital. The benefits of this methodology include:

- The ability to determine optimized presurgical planning, here there is the possibility for effective planning, which can save time and lower the potential risk for patients.
- Improving patient communication through the demonstration of operating procedures.
- Achieving reduced lead time for the fabrication of models, patient-specific prosthesis and surgical instruments. POC fabrication is as a result optimum for creating accurate models and devices when required.
- Improved procedure outcomes as medical professionals, designs, technicians, and engineers can develop novel care solutions and technologies.

6.5 Patient-specific implants

The most prominent use of 3D printing in medicine and surgery is that of the ability to manufacture patient specific implants. Because scans are taken from a patient, then implants produced can be made to be identical to the size and shape required. An implant can be matched to the specific needs of a particular patient. Fig. 6.11 shows the fabrication of a portion of lateral meniscus disc. This has been 3D bioprinted using

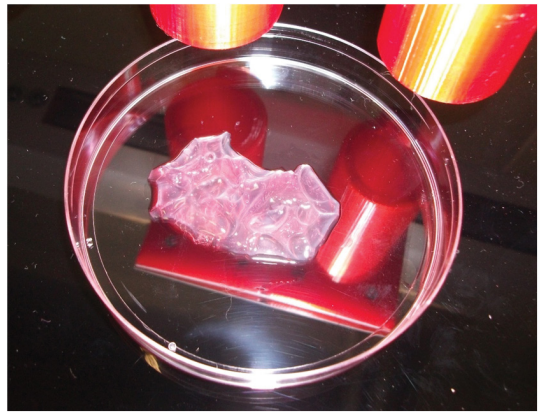

Figure 6.11 The fabrication of portion of a lateral meniscus disc 3D bioprinted in 11 layers.

Figure 6.12 3D design 3D printable knee replacement implant, this is designed to be fabricated using SLS printing to generate a phosphate composite polymer structure.

a biogel that can mature into a cartilage implant. Although in its infancy, bioprinting technology is an exciting example of new techniques that can have great potential to patients.

Implants technology continues to develop with innovative solutions allowing the integration of integrated structures and connecting tissues. As a result, there is the ability to increase tissue function [17,18]. However, the development of newer implants requires that technical and medical domains work together. This is particularly when materials science, engineering, surgery, and mechanical engineering disciplines work together.

3D printed soft tissue implants that have biocompatible and bioactive properties can also be developed. As a result, implants can be engineered to promote tissue regeneration and integration of the implant with the surrounding tissue.

Fig. 6.12 shows the design of a patient specific knee implant that can be 3D printed from calcium phosphate composite polymer. It is only through the integration of CT, scans, software rebuilds, materials science, and effective surgical procedure, that can ensure the fabrication of parts that are enhanced over the status quo.

The implant shown in Fig. 6.15 were designed in Autodesk Inventor computer-aided design software. It was an iterative design that was improved over the current model. The first prototype was 3D printed using SLS and fitted into place on a surgical model of the patient's knee. It is through the 3D printing fabrication and using computer aided design that the optimum device can be fabricated. Here, newer materials were investigated to test the possibility for biological integration.

It is the physical interaction of implants and the connecting tissues around the implant, which can cause complications. The implant tissue surface interface is as a result an important part of the implant structure.

This has been taken one step further with the fabrication of biological issue systems as shown in Fig. 6.13. A problem with current metallic and polymer implants is that

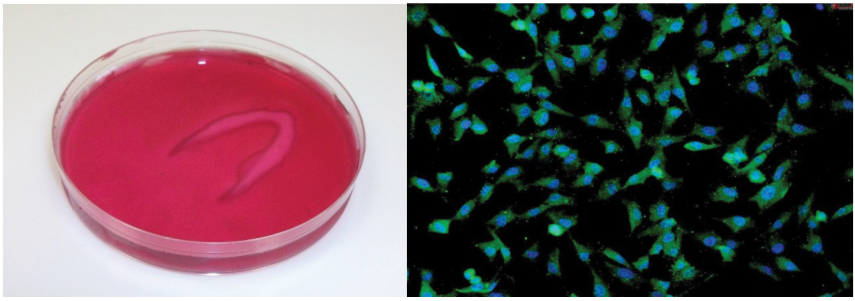

Figure 6.13 (left) a 3D bioprinted medial meniscus in a petri dish of cell culture medium and (right) fluorescent scan of chondrocyte cells postbioprinting.

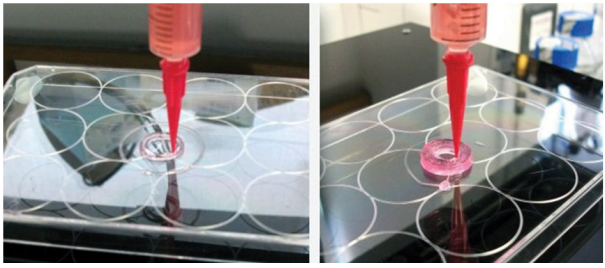

Figure 6.14 3D bioprinting of a nipple and areola complex (left) layer 3 being generated and (right) layer 18 being generated.

they can failure over time because of fatigue. It can also cause cell death due to the movement of the implant across the tissue interface. It is therefore important to further develop technologies that connect a tissue. 3D bioprinted structures as a result allow the fabrication of direct like-for-like tissues.

Calcium phosphate implants as were as cartilage implants are new possible. These can also be used in regions where mechanical support is required. Cell proliferation has also been demonstrated together with biocompatibility and mechanical stability.

Fig. 6.14 shows the fabrication of a nipple and areola complex through the process of 3D bioprinting. Here, an alginate-based hydrogel is 3D printed to form a 3D structure. The alginate hydrogel contains 3 million chondrocyte cells per 1 mL. Following the bioprinting process, the structure is cross-linked with calcium chloride. In order for the hydrogel structure, then there is the requirement for incubation and mechanical stimulation. This process is shown in Fig. 6.15. Rotational mechanical stimulation has been found to offer the optimum formation of a tissue structures that have the required mechanical properties.

3D printed implants offer a significant potential for restoring on enhancing bodily function. Particularly, this is the case on patients with orthopedic injuries. In order to rebuild such structures, then it is possible to produce large orthopedic structures as shown in Fig. 6.16. The rebuild of the complete structure was made by mirroring the

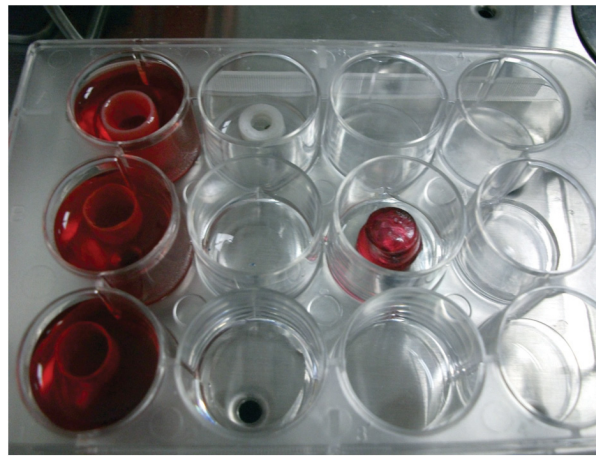

Figure 6.15 Postfabrication testing of a series of 3D printed vascular grafts in the left-hand side wells, the center top well has a small 3D printed bone structure and the right-hand side well has a nipple and areola complex bioprinted tissue.

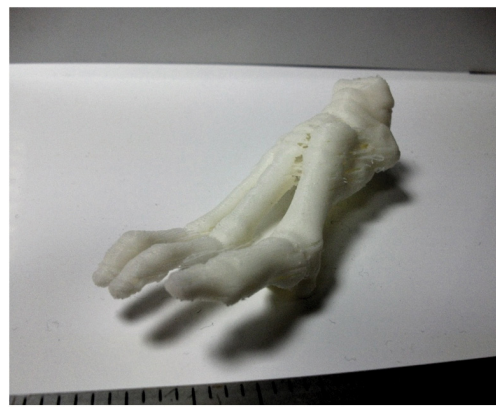

Figure 6.16 The generation of the whole foot structure, this was 3D printed from PCL biocompatible monomer.

patients opposite foot. Slight modifications needed to be made using computer-aided design software. The whole structure was subsequently 3D printed from polycaprolactone (PCL).

Orthopedic implants such as this need to both integrate with and regenerate a whole bone structure. Subsequently, replacement tissue can form over the implant to produce a mechanically supportive structure. In order to promote bone regeneration, then the structure also needs to be porous so that cell proliferation can be achieved toward the inner part of the implant structure.

3D printing of bioabsorbable and osteoinductive materials, such as calcium phosphate PCL is effective in promoting bone growth [19]. There is also the requirement

to lace the implant with growth factors such as bone morphogenetic protein. Although it might not yet be feasible to regenerate a transplant a whole complex bone structure, it is possible to produce small bone structures to repair significant damage to a bone that cannot be repaired using conventional technologies.

Despite the promises of 3D printing technology, there are some limitations to its wider adoption. A key consideration is both the expense and time required to produce a model, implant, or surgical instrument. There is still a high degree of skill required to generate 3D printed parts that are accurate and to the requirements of a surgeon or medical practitioner.

6.6 Conclusions

This chapter demonstrates that there are indeed significant benefits for using 3D printing for the generation of both surgical implants and instruments. As 3D printing technologies advance, the speed, quality, and range of materials will increase. This is particularly the case with the development of 3D printable biocompatible materials that can extend capabilities. The use of these is particularly important for tissue-repair applications and in augmenting surgery. Further still, 3D bioprinting is an important application that may again reduce surgical complications by generating patient specific tissues. With all this said, 3D printing will have a significant impact on the future of healthcare.

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Part 2

Applications of 3D Printing in Transplantation Procedures

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3D printing in dental implants

7

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Chapter outline

7.1 Introduction

The most common reason behind teeth loss is periodontitis (infection and inflammation of the gums and supporting structure of the teeth), and other causes include dental caries, trauma, developmental defects, and genetic disorders. The use of dental implants with varied compositions to rehabilitate the loss of teeth is a general practice, which has been universally followed. Great deal of basic and clinical research has been employed in the development of various dental prostheses that will cope with the consequences of partial and complete edentulism. Edentulism is a debilitating and irreversible condition and is often described as the "final marker of disease burden for oral health" [1]. Edentulism leads to several oral consequences leading to impaired masticatory function and unhealthy diet [2]. Thus, the dental science (or dentistry) plays a prominent role in maintaining proper oral health. It has a long history of con-

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tributing to the needs of patients by offering dental restoration and prosthetic devices (like inlays, onlays, crowns, removable dentures, and fixed partial dentures) due to a never-ending demand in dentistry, which flourished several new materials and technologies over decades. Dental drilling was started soon after the invention of anesthetics, which helped in the teeth-filling of materials (like silicates and amalgams) with wider acceptability. Various acrylic resins for dentures, and acidic monomers and polymers for restorative composite materials preparation have been bloomed during the 1940s–1950s. The discovery by Swedish physician Per-Ingvar Branemark (touted as the father of modern dental implantology) of the exceptional features of titanium metal explored its translational application in dental implantology. Later in the 20th century, loss-wax casting process to dentistry was introduced for the construction of crowns and bridges. These are well-established conventional dental laboratory technologies. However, vast array of versatile implants still faces a major problem with the rate of degradation and assembly at the site of implant. Therefore, the increased demand for safe and aesthetical dental materials has been anticipated by sophisticated manufacturing technology. In this context, the search for alternatives to conventional treatment strategies for the repair or replacement of malfunctioning dental structures, additive manufacturing (AM), or 3D printing emerged to be a promising technology. This chapter deals with the use of 3D printing technology for the production of new dental implants for overcoming the existing dental problems.

7.2 Dental implants

Dental implants are the materials used as artificial tooth roots, which are similar in shape to screws and are placed in jawbone. Dental implants have become a very popular solution toward common and sometimes most unique dental problems due to the high success rate and predictability of the procedure. Moreover, relatively less medical complications during the treatment made this procedure highly accessible to the general population. Dental implants overcome the drawbacks of conventional fixed partial denture in terms of higher success rate and decreased sensitivity to adjacent tooth [3]. The history of dental implantation is depicted in Fig. 7.1. The International Congress of Oral Implantologists (ICOI) developed the "Pisa Implant Quality of Health Scale," enlisted dental implants into four categories from optimum to clinical failure or absolute failure based on levels of sensitivity, mobility, bone loss, and presence of exudates [4]. A successful dental implant should consider the points during its development as well as its usage as depicted in Fig. 7.2. Dental implants are classified into endosteal implants [5] and subperiosteal implants [6] based on the positioning of implants. Endosteal implants are implanted surgically into the jawbone followed by the attachment of an artificial tooth once gum is healed. While on the other hand, subperiosteal implants involve the attachment of metal frame onto the jawbone with a post that protrudes from the gum to which artificial tooth is implanted. Both these implants need to accomplish the criteria of successful dental implantation with lesser pain and zero mobility.

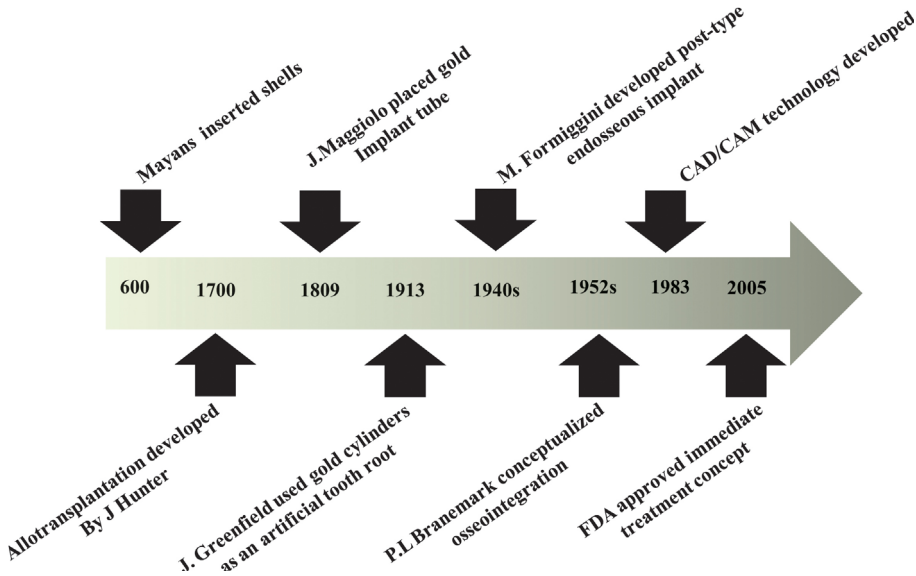

Figure 7.1 History of dental implantation.

Figure 7.2 Criteria for successful dental implant.

Dental implants are generally composite alloplastic biomaterials, polymer-coated metallic (like titanium) implants, tissue-engineered implants, and functionally graded materials (FGM; material with changing composition, microstructure, or porosity across the volume of that material). Most dental implants clinically used today are made from commercially pure titanium, Ti–Al–V alloys, and so on, which provide mechanical properties under loading. The selection of the materials is based on their degree of biocompatibility and resistance toward corrosion. But many of these materials undergo material failure under long-term physiological strain leading to the surgical removal of implants. Design of a dental implant generally depends upon implant length, implant diameter, shape, surface characteristics, and mechanical strength. For an effective dental implant, the estimated length and diameter should be in the range of 8–15 mm and 3.25–6 mm, respectively. Hollow cylinders, solid cylinders, and screws are considered to be the ideal shapes for dental implants with modulated surfaces. There are several aspects to be taken into account for ensuring successful dental implantation and avoiding complications associated with it [7]. Altogether, the goal of a successful implant design is to best anchor the implant into the bony ridge so as to facilitate easy and painless procedures.

Currently used dental implants include carbon implant, blade-vent implant, single-crystal sapphire implant, aluminum ceramic implant, TPS screw, ITI hollow-cylinder implant, IMZ dental implant, core-vent titanium alloy implant, transosteal mandibular staple bone plate, and osteo-integrated titanium implant [8]. Carbon implant is considered to be a permanent or prolonged dental prosthesis in a jawbone comprising a carbon root and a base portion for insertion into a mandible jaw socket. Biologically inert carbon root with a thickness of about 0.1–1 mm is said to permit attachment of tissue [9]. However, it was reported to fail in certain patients with the emergence of osteomyelitis and paresthesia after using vitreous carbon implant [10]. Ceramic formulations including hydroxyapatite were involved in initial adhesion of bone-forming cells for successful osteo-integration during the treatment. Thomas J. Webster has developed a nanostructured ceramic (grain size of 1–100 nm)-based nanocomposite with an adhesion-promoting peptide and a nonpeptide polymer for enhancing osteoblast functions on a surface of a dental implant. These composites claim to aid in the formation of new bone at the tissue/biomaterial interface and therefore improve dental implant efficacy [11]. Similarly, the rate of osteo-integration can be increased by surface functionalization of the metal surface of titanium dental implant using gold nanoparticles in the presence of (3-mercaptopropyl) trimethoxysilane. Surface-functionalized titanium implants proved to promote osteogenic differentiation of human adipose-derived stem cells in both in vitro and in vivo systems [12].

7.3 3D printing

More than three decades of 3D printing journey was begun with an invention of stereolithography by Chuck Hull [13]. Chuck and 3D Systems have made significant contribution in filling the gap of communication between computer and rapid prototyping methods by the development of ".stl" file format in computer-aided designing (CAD) software, which is still in use for guiding the printer to print a 3D object, commonly

termed as computer-aided manufacturing (CAM). The continuous technological advances are being implemented in 3D printing for realizing the biomedical challenges that are involved in the printing of complex biological designs (Fig. 7.3).

3D printing is a quick prototyping and additive manufacturing practice often engaged in the fabrication of complex architecture with extraordinary precision through a layer-by-layer building using a step-by-step process. The 3D printing of any material involves a series of procedures as shown in Fig. 7.4. Additive manufacturing system consists of majorly three components, which are (1) scanning/digitization tool,

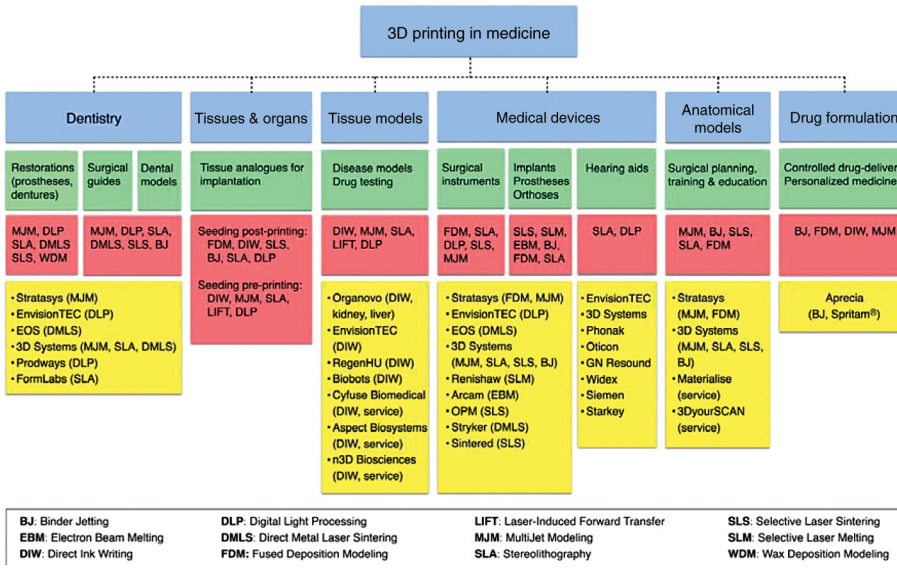

Figure 7.3 3D printing hierarchy in medicine shows its application areas (*blue boxes*) with respective 3D-printed products (*green boxes*), primary 3D printing technologies (*red boxes*) and key technology and service providers (*yellow boxes*).
Source: Reproduced with permission from Liaw et al. (2017).

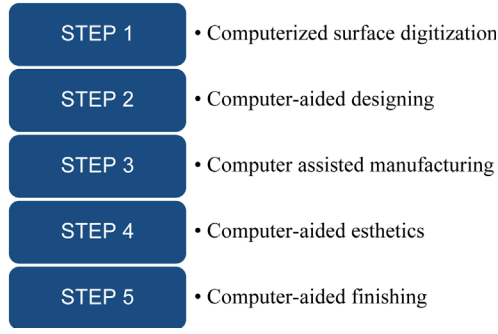

Figure 7.4 Steps in CAD/CAM technology for the fabrication of 3D prosthesis.

(2) software, and (3) a manufacturing scanner (optical or mechanical) system. This programmed process facilitates the fabrication of 3D products having precisely controlled architecture with high reproducibility and repeatability. 3D printing describes a manufacturing approach using polymeric solutions that builds prosthetics one layer at a time, adding numerous layers to form a 3D construct [14].

7.4 Materials in 3D printing of dental implants

3D printing holds diverse applicability in dentistry with a great deal of promise to make new and exciting treatments and approaches in manufacturing for dental restorations. Materials used in 3D printing are required to satisfy certain criteria for aesthetic construction of 3D implants in dentistry. First, the biocompatible nature of the material is essential for the coexistence of the graft with the current histological status of the recipient. This complements the interaction of implanted material to the surrounding gingival tissue as well as the immune system of the personnel. Second, the printability status of the material attributes to the role of these in producing perfect temporal construction of the grafts with lesser effort. The printability relates with the category of bio-printing technique required for the implant preparation, for instance, microextrusion technique [15] relies on the viscosity of the material for the formation of rigid constructs. Third, materials to be selected with appropriate mechanical properties sustain the force applied on the implant [16]. Many instrumentation facilities are available nowadays to criss-cross the impact of applied strain on the implant and one such instrument is Universal Testing Machine (UTM) [17]. UTM can be used for the evaluation of tensile strength and compressive strength of materials, which is necessary for the creation of efficient dental implant. Finally, the biodegradability of the implant is far important for replacing the material with their extracellular matrix proteins for appreciable process of implantation [18]. The concept of functionally graded material (FGM) originated in Japan in 1984, where it is proposed as a material for thermal barrier to the surface of space plane that can withstand a surface temperature of 2000 K with a temperature gradient of 1000 K across a 10-mm cross-section of material [19]. Later, in 1986, FGM was further represented as a potential material for bone reconstruction to replace the conventional implant material. Functionally graded material (FGM) represented initially in Japan (1986) for the replacement of conventional implant materials for bone reconstruction. Usually, small-size scaffolds or polymers are widely used for dental implant production that provides strong masticatory function after tooth loss [20]. FGM provides higher rate of biocompatibility, decreased stress effect, lesser mechanical failure, and improved biodegradability to the implant. FGM dental implant comprises of a cylindrical structure with strong upper part and highly degradable lower site. This construct helps in providing the occlusal force applied on the top that gets transmitted directly to the bottom of the implant placed in the gum. Hydroxyapatite (HA) with titanium (Ti) is considered to be a good amalgam fabricating a dental implant having a great mechanical reinforcement. The successful reduction of maximum stress in bone can be achieved using graded elastic moduli of coating material with titanium, adjacent to the bone, this cause reduction in stress,

and can prevent slight fracture and fatigue failure of the bone. Similarly, the graded elastic moduli of coating material from HA can improve biocompatibility and achieve complete osteogenesis at the site of tooth loss. Also, the increased thickness of coating material can reduce the stress concentration in the bone for most of the FGMs [21].

Guo et al. reported the preparation of HA/ZrO_2 FGM for dental implants, which demonstrated higher thermo-compatibility and greater durability for the clinical practice [22]. These FGMs were constructed using spark plasma sintering (SPS) method, which allowed equiaxial deposition of zirconia grains on HA matrix, resulting in increased tensile strength of the implant. Ti/SiO_2 FGM dental implants were manufactured by CIP (cold isostatic pressing) method and then sintered in argon gas that provide higher values of elastic modulus and strain [23]. Porous FGM dental implant can be manufactured based on the partial densification during metal powder sintering or powder metallurgy. Laser-Engineered Net Shaping (LENS) is a novel technique for the production of porous dental implants with mechanical properties matching those of natural bone. An increase in Young's modulus with increasing density of the material can be observed in this technique [24].

7.5 3D printing in dental implants

3D printing technology has effectively been tapped for the synthesis of novel dental implants with both porous and roughened surfaces. However, it involves several steps to produce efficient dental implant with complex geometry for successful dental restoration. These include analyzing dental deformations, designing by CAD technology, selection of cell or polymeric material for manufacturing, bio-printing, and implantation.

The contribution of additive manufacturing in dentistry started during 1980s. Since then, this CAD/CAM technology has evolved into two major directions: (1) intraoperatory application for one appointment restoration and (2) emergence and expansion of AM technology (CAD/CAM systems and related materials) to commercial production centers and dental laboratories for restorative production. In particular, three pioneers have changed the treatment strategies by significant development in the dentistry using additive manufacturing. In the series of development, Dr. Duret was the first, who began the fabrication of functional optical crowns using a series of systems that initiated with an optical impression of the abutment tooth. Later, he and his colleagues popularized AM in dentistry by developing commercial Sopha System [25,26]. The second person is a dentist, Dr. Mörmann, who is the pioneer of CEREC method. The state-of-the-art CEREC system was developed in 1985 at the University of Zurich in Switzerland. CEREC stands for “Chair-side Economical Restorations of Esthetic Ceramic.” It is considered to be the world's only chair side dental CAD/CAM restorative system. Unlike traditional impression of tooth, CEREC used digital camera picture and convert to a 3D virtual model of the prepped tooth using CEREC 3D software. This software helps dentist to design a tooth restoration (crowns, inlays, onlays, or veneers) that usually requires about 5 min. Later, the design can be communicated to CEREC unit for manufacturing.

Nickel–chromium-based alloys were used as a substitute for gold in dentistry because of the lofty increase of gold prices during 1980s. However, due to the metal allergies especially in northern Europe, adaptation of allergy-free titanium was advocated. Since the precision casting of titanium was still difficult at that time, therefore, a dentist, Dr. Andersson, the third person who attempted fabrication of titanium copings by spark erosion. He introduced the CAD/CAM technology-based processing of composite veneered restorations and developed Procera system in 1983 for high-precision, repeatable manufacturing of dental crowns. This system further developed as a processing center that networked with satellite digitizers around the globe for the synthesis of all type of ceramic frameworks, and are adopted by a number of companies globally. Imaging of dental deformities involves different diagnostic technologies like X-ray and CT imaging that guide the synthesis of proper dental implant. Dental radiographs (X-ray images) provide the attributes that contribute to the dental defects in the region of gum during edentulism while CT imaging gives idea on 3D construction of the region of defect. These X-rays are used with low levels of radiation to capture images of the interior of the teeth and gums. The X-ray unit is placed close to the gum region with minimal film-focus distance to undertake dental radiography in patients. One of the most promising technologies for dental restoration involves CAD/CAM (computer-aided design and computer-aided manufacturing) technology in modern era. The development of CAD/CAM is based around three elements, namely: (1) data acquisition, (2) data processing, and (3) manufacturing. Dental CAD/CAM systems consist of a handheld scanner, which houses a computer together with a monitor, and a milling machine, which provides 2D and 3D images for tooth preparation. For the past 25 years, approximately 2000 restorations have been found to possess higher success rate in postimplantation survey. Lava Chairside Oral Scanner is one of the CAD systems that provides both 2D as well as 3D images before dental restoration [25]. With the aid of optical or laboratory scanners, a precise virtual model can be developed for crown copings and dental frameworks [27–29]. 3D printing may be harnessed for the fabrication of metal structures by printing either directly or indirectly in burn-out resins. Restorative dentistry such as veneering material addition may require the construction of master model necessary for further fabrication [30]. In 2017, additive manufacturing becomes mainstream in the dentistry. According to the latest report from SmarTech Publishing [31] (https://www.smartechpublishing.com/news/dental-3d-printing-market), the dental 3D printing market will cross the market growth over 9 billion USD by 2027 (Fig. 7.5).

7.6 Techniques in 3D printing and application in dentistry

3D printing encompasses different techniques for efficient production of biocompatible and stable constructs. These include stereolithography, inkjet bio-printing, photopolymer jetting, powder-based 3D printing, and direct metal laser sintering.

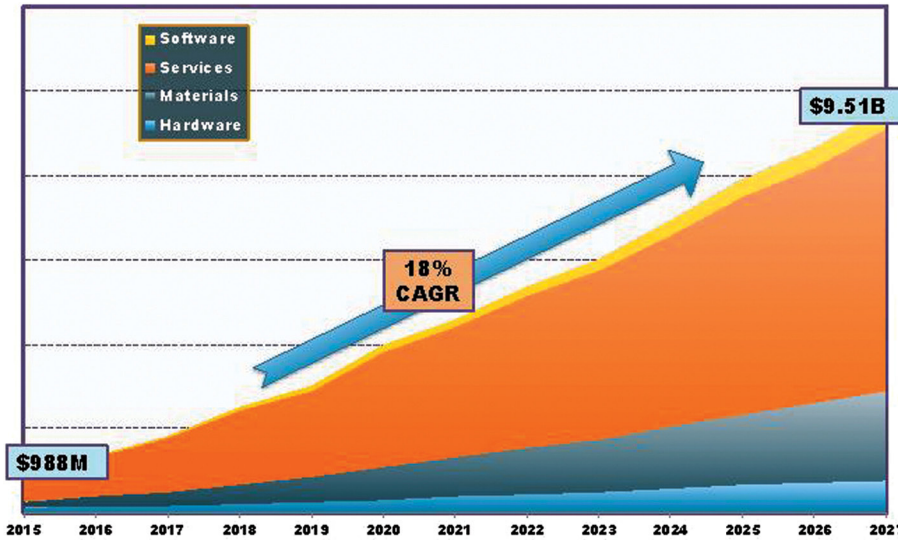

Figure 7.5 Forecast on categorized dental 3D printing revenue generation from 2015 to 2027 by SmarTech Publishing.
Source: SmarTech Publishing 3D Printing in Dentistry 2018.

7.6.1 Stereolithography

Stereolithography uses a stereolithography apparatus (SLA), which converts liquid plastic into solid objects [32]. This is an early and widely used 3D printing technology. In the early 1980s, Japanese researcher Hideo Kodama first invented the modern layered approach to stereolithography using UV light for curing of photo-hardening thermoset polymer [33]. However, the term "stereolithography" was first introduced in 1984 by Charles W. Hull when he filed patent for the process [13]. He defined it as a method for making solid objects by successively printing thin layers of an ultraviolet curable material one on top of the other [34] (Fig. 7.6).

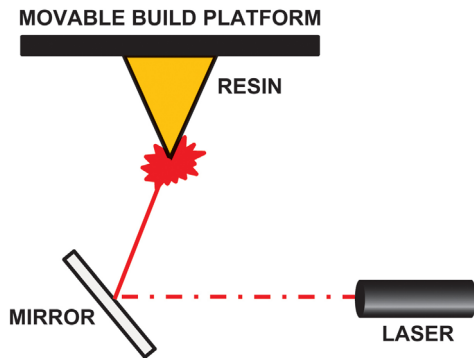

Figure 7.6 Stereolithography.

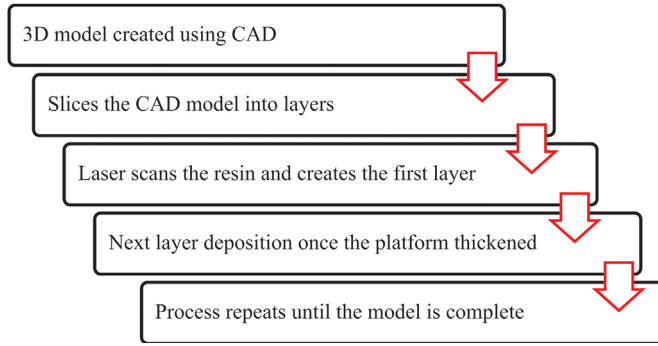

Figure 7.7 Basic manufacturing process for 3D printing.

In this technique, CAD files get translated in to Standard Tessellation Language (STL) files that recruit the production of 3D printed objects (Fig. 7.7). Stereolithography has become a most popular technique in the rapid prototyping sector and is the first commercially available rapid prototype. In preoperative modeling and surgery simulation, stereolithography has been used in various medical fields like reconstructive surgery, tumor surgery, craniofacial surgery, preprosthetic surgery, orthognathic surgery, and dental implants [35–40]. This technique is limited as it can use only one resin at a time, and the resins are often either epoxy-based or acrylic. High cost of raw materials and machine maintenance makes this technique less cost efficient than other 3D printing techniques. This technique is labor intensive as it needs manual postbuild handling, thus making it time-consuming as well [41] as shortcoming its faster clinical translation. Eventually, different other techniques are also being developed for overcoming the issues associated with old approach [42]. In 1991, human anatomy models are produced by stereolithography technology and were first used in a maxillofacial surgery clinic in Vienna [43]. In September 2008, maxillary and mandibular malocclusion of a 39-year-old woman was corrected using implant surgery with the aid of tooth supported surgical template fabricated by stereolithography at the department of orthodontics, Tokyo Dental College Chiba Hospital. In this study, preoperative simulation was fabricated using SLA followed by its implantation with 5 years of complete success rate with no side effects [44].

A modified SLA system called continuous liquid interface production (CLIP) simplifies traditional SLA by creating a persistent liquid interface with oxygen-permeable window below the UV image project plane, thus increasing the production speed. This technology was invented by Joseph DeSimone and his colleagues, originally owned by EiPi Systems. But now it is being developed by Carbon 3D Inc., founded by Joseph DeSimone and Philip DeSimone in December 2013. Briefly, CLIP technology involves a photochemical process that harnesses light and oxygen to rapidly produce objects from a pool of resin. Carbon 3D Inc. is advancing the 3D printing technology to serve producer for possible production of future materials from everyday products like tennis shoes and electronics, to industrial components, to highly customizable medical devices (Fig. 7.8) (https://www.carbon3d.com/industry/dental-materials) [45]. CLIP-based Carbon's Digital Light Synthesis technology can be used by dental

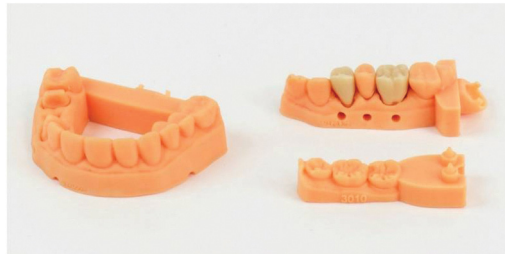

Figure 7.8 Dental model fabricated using the Continuous Liquid Interface Production (CLIP) methodology of 3D printing developed by Carbon3D Inc.
Source: Carbon3D Inc.

and orthodontic laboratories for quick production of 3D printed dentures with base and teeth. These are commercially known as DENTCA dentures for Carbon printers, sold by DENTCA, and are claimed as first FDA cleared 3D printed dentures.

7.6.2 Inkjet bioprinting

Direct inkjet printing (DIP) has an advantage over other technologies referred over here that it can produce dense structures with complex architecture while the other methods can create only porous structures. In 3D inkjet printing, a layer of powder is evenly placed on a stage and droplets of binding agent are printed onto the surface for solidification (Fig. 7.9). Ebert et al. used DIP in making zirconia prosthesis for dental implants using a drop on demand inkjet printer in the size of a posterior crown [46]. The process had high precision and minimum material consumption. In this, ink droplets are generated either using heat or transient pressure that allows the ink to pass out of the nozzle onto the substrates. However, the droplets created by heat are uneven, unmixed, and produce rough surfaces while other means produce directional printing

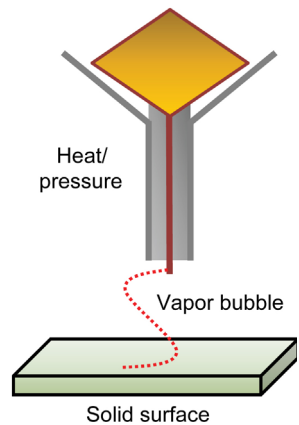

Figure 7.9 Inkjet bio printing.

with regular and equal size [34]. This technology is employed for the development of porous calcium polyphosphate (CPP) structures for tissue engineering. University of Sheffield used this method to develop colored soft tissue prostheses. So far, this technique is not practically used for the construction of dental implants but it can be employed for the creation of dental prostheses in dentistry applications [47]. However, very often used polymer glues are heavily toxic in nature, which limits the suitability of these fabricated materials for biological applications.

Özkol et al. have recently investigated the potential use of direct inkjet printing method for manufacturing 3% Yttria stabilized tetragonal zirconia polycrystals (3Y-TZP) dental restorations [48]. He used aqueous inks of 3Y-TZP and carbon for producing a sample 3Y-TZP framework of a dental bridge in a millimeter scale using DIP. The four-point bending test showed higher flexural strength (~843 MPa) of 3Y-TZP components than slip cast 3Y-TZP components (~684 MPa). The fabricated ceramic components showed a smooth surface without the stair steps effect and drying or sintering cracks. This study suggested that a relative density of >96% of the theoretical density can be achieved in 3D printed materials, thus promoting DIP method as a potential and promising fabrication technique for manufacturing dental restorations (Fig. 7.10).

7.6.3 Photopolymer jetting

Photopolymer jetting (commonly known as PolyJet technology) developed by Israel-based company "Objet Geometries Ltd.," was later merged with "Stratasys" (a well-known leading manufacturer of 3D printers) in 2012. The PolyJet technology

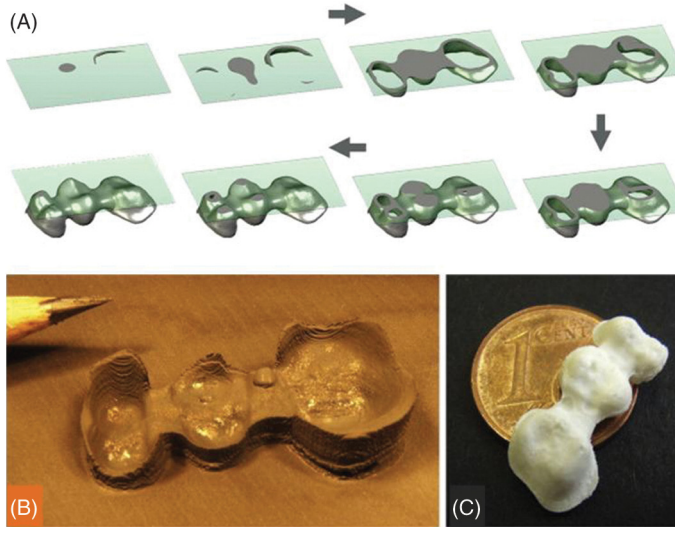

Figure 7.10 Use of Direct Inkjet Printing (DIP) manufacturing. Image (A) shows that the cross-section varies throughout the object for the visualization of the layer-wise stages of the printed object. Image (B) represents the supportive base made of carbon black and image (C) shows the final sintered 3Y-TZP bridge framework.
Source: Reproduced with permission from Özkol et al. [48].

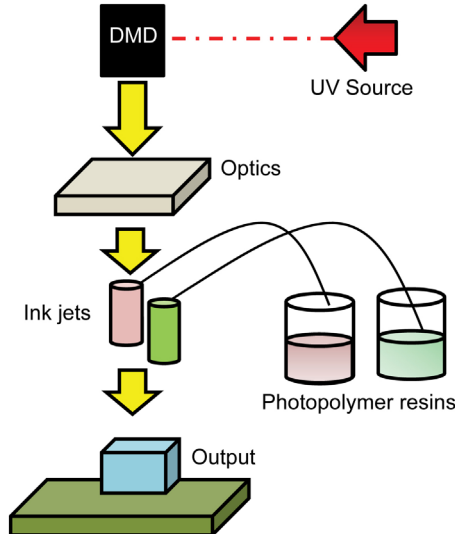

Figure 7.11 Photopolymer jetting.

involves the use of liquid photopolymers in order to create a solid 3D object (Fig. 7.11). This type of printer consists of jetting heads, which moves back and forth in *X*-axis and draws a layer of photopolymer on a platform followed by instant UV curing of each layer in the 3D printer and thus creates an accurate model with excellent surface finishing [49]. Among the jetting heads of PolyJet printer, one head is meant for desired product and the other head is for supporting materials. Once the printing of 3D object is completed, water jet removes supporting material easily.

Interestingly, the designed products can be used instantly without postcuring processing. Due to the use of UV energy for curing the photopolymer materials in both stereolithography and PolyJet techniques are sometimes misunderstood to be a same technology. However, few important features, as listed in Table 7.1, separate these two 3D printing processes from each other, which is important to know for choosing the best process for specific manufacturing.

Table 7.1 Principle differences in the stereolithography and PolyJet 3D printing technology.

Features	Stereolithography	PolyJet
Curing method	UV lasers directed via dynamic mirrors onto a bed of liquid photopolymers for curing	Curing of materials after deposition of liquid photopolymer on build platform
Object recovery	Hand sanding, light bead blasting	Water blasting, some residue removal by hand
Resolution	Good @ 0.005–0.002”	Excellent @ 0.00063”
Ideal size	Large prototypes and master patterns	Small designs with highly detailed prototypes and master patterns
Optical property	Generally opaque	Opaque, multicolor, transparent

Practically, PolyJet printers are generating great progress in printing because of applying multiple materials at the same time using multiple print heads and places for multiple materials. This multifunctional feature opens the door to produce variety of materials at a single time resulting in increase of production volume (Lipson and Kurman, 2013). Biocompatible (MED 610), VeroDentPlus (MED 690), and VeroDent (MED 670) are some of the dental restoration materials producing by Stratasys using PolyJet technology.

Mai et al. fabricated fit of interim crowns using photopolymer-jetting 3D printing and compared it with that of milling and compression molding methods. In this, a virtual crown was designed using 60 μm cementation spaces and transferred to PolyJet 3D printer. The interim crowns were fabricated by printing a biocompatible photopolymer resin with a layering thickness of 5 μm, and later the constructs were rinsed with running water and soaked in isopropanol. The absolute marginal discrepancy was found to be smallest in the PolyJet group at 99 ± 19 μm compared to molding method. This technique improves the fit of interim restorations with better biological and mechanical functions replacing the currently available expensive implants [50] (Fig. 7.12).

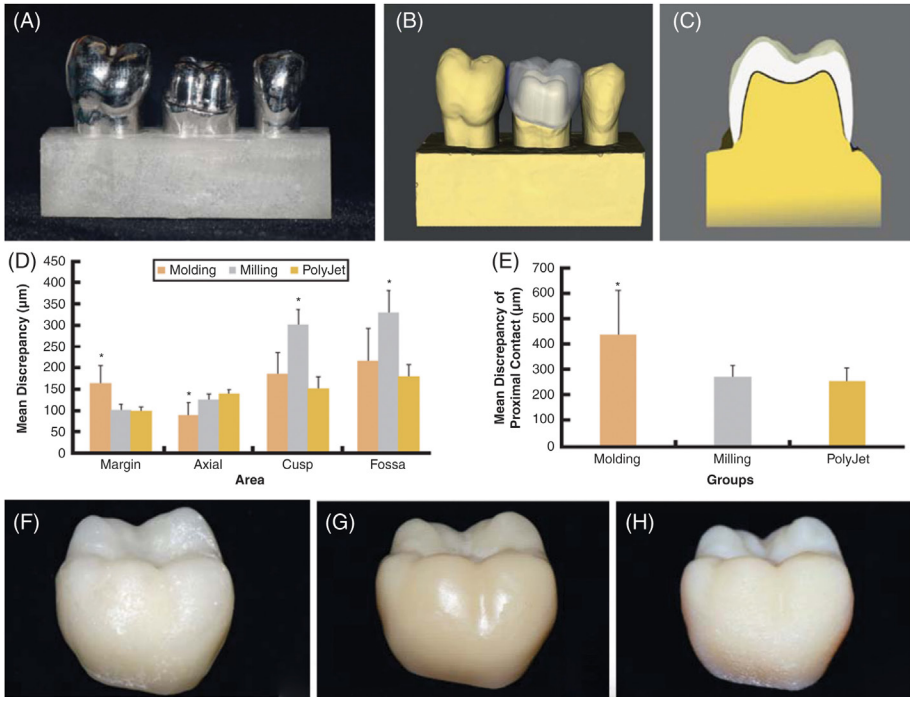

Figure 7.12 (A) Master model with metal dies embedded in custom resin base; interim crown design. (B) Overall contour. (C) Cross-sectional image in buccolingual direction. (D) Mean marginal and internal discrepancies of restoration. (E) Mean discrepancy of proximal contact points. Interim crowns fabricated with different methods: (F) molding, (G) milling, (H) PolyJet. *Source*: Reproduced with permission from Mai et al. [50].

7.6.4 Powder binder 3D printing

Powder binder 3D printing uses a modified inkjet head and print a pigmented liquid onto the powder by layer-by-layer approach. It is quite similar to laser sintering, however, a binding agent is placed onto the powdered thin layer and it continues until the complete model is achieved [51]. Tamini et al., constructed a monolithic monetite onlays using dicalcium phosphate dehydrate using a 3D-powder printing system followed by the construction of monolithic blocks using CAD software. The bone formation process was monitored by PET-CT and found bone mineralization after placement of the onlays. The results demonstrated the possibility of Osseo integration of dental implants in bone augmented with synthetic monetite onlays. Increased bone formation was found in onlays with higher rate of porosity [52] (Fig. 7.13).

Materials with controlled and variable porosity can reduce the imbalance between different stress modulus of bone tissues and implants, thus promoting long-term fixation and stability. A study was conducted with a 3-year follow-up in order to evaluate the survival and success rate of single 3D printed/additive manufacturing titanium implants placed in both jaws. The investigation enrolls all patients with a single-tooth gap or unrecoverable tooth with good oral health and excludes those with poor oral hygiene and bruxism. Tixos dental implants were fabricated with an AM technology from powders of titanium alloy (Ti–6Al–V) using Yb (ytterbium) fiber laser system. About 110 a.m. implants were installed in healed ridges and postextraction sockets, out of which only six implants failed after 3 years of functional loading with a success rate of 94.3%, which suggests the real potential of 3D printing implants in restoring edentulous arches with long-term performance and mechanical resistance [53].

7.6.5 Direct metal laser sintering

Direct Metal Laser Sintering (DMLS) is the most advanced technique in 3D printing technology when the manufacturing protocol depends on the 3D printing of metal implants. In this method, metals with uniform dimensions were allowed to form 3D structures using layer-by-layer approach in the presence of a laser [54] (Fig. 7.14). In this process, laser hits the powder to create a melt pool and then the particles fuse together and form a layer. After each layer, the powder bed is lowered by one layer thickness to allow a new layer applied on top of it till the material structure is completed. This technology has wider acceptability around the globe due to its ability to fabricate complex geometries directly from the CAD digital data. The used terminology for this approach could be confusing due to nonobvious differentiation between the various techniques. For example, use of ceramic and polymers is generally referred as selective laser sintering, while use of metal is cited as direct metal laser sintering in the industrial culture.

A pilot study was conducted at McMaster University used DMLS for the osseointegration of dual-stemmed shaped dental implant using Ti–6Al–V powder (SIT implants). The novel design with significant geometrical change provides prong-like stems in contrast with conventional implants. Osteoclast- and osteoblast-mediated

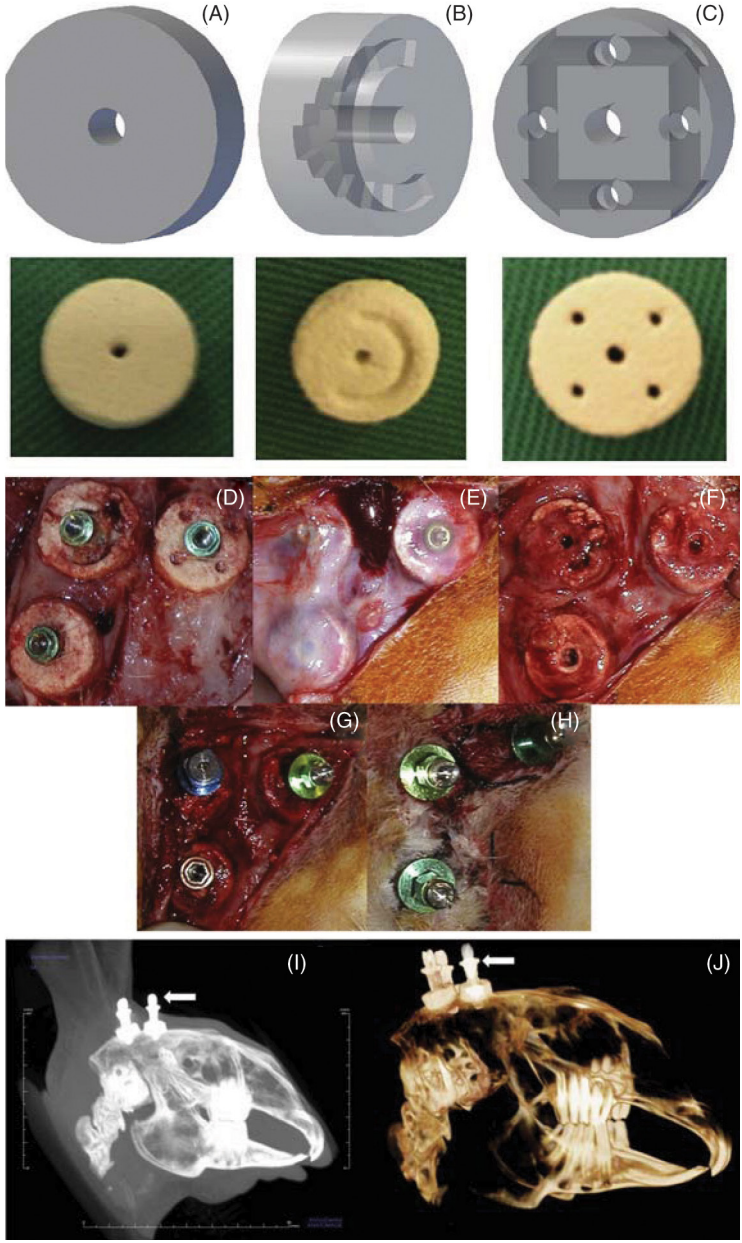

Figure 7.13 CAD images of the onlay designs (top) compared with photographs of the 3D-printed monetite bioceramics (bottom): (A) Design A, monolithic without any surface modifications; (B) designs B and C had a C-shaped groove either on the superior surface of the on lay facing the periosteum (design B) or on the inferior surface of the on lay facing the bone (design C); (C) design D had 8 interconnected channels (4 vertical and 4 horizontal) opened into all the surfaces of the on lay. All designs possessed a central hole to allow placement of osteosynthesis screws. Photographs depicting the surgical procedure: (D) onlay placement fixation with osteosynthesis screws; (E) Opening of the surgical sites after 4 weeks; (F) removal of the osteosynthesis screws; (G) implant placement in the holes left to be the removed screws; (H) suturing of the surgical site; (I, J) CT scan and cone beam in a lateral view of the skull showing the Ti implants (*arrows*) in the monetite onlays following placement on the rabbit calvaria. *Source*: Reproduced with permission from Tamimi et al. [52].

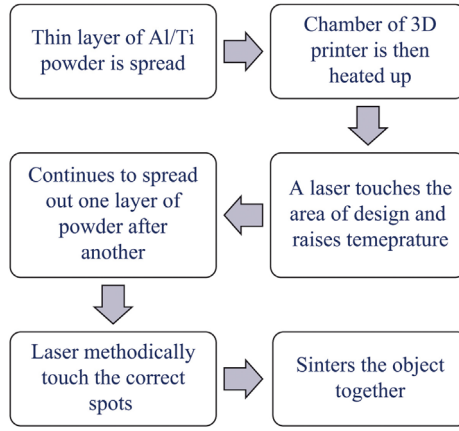

Figure 7.14 Work flow chart of Direct Metal Laser Sintering (DMLS).

bone matrix resorption and deposition were monitored for both SIT and control implants, which showed active bone growth and remodeling within a period of 12 weeks in rabbits. The use of micro-CT enables the visualization of implant and cortical bone volume while the extent of bone growth from the cortical region down the implant surface was monitored by SEM. The study proposes the titanium SIT implant as a better conductor of bone growth at early and late time points with lesser rate of stress cracking and bone debris [55]. One-year multicenter study was done by Mangano et al. on 201 DMLS titanium implants including 95 mandible and 106 maxillae. A success criterion was evaluated that includes absence of pain, sensitivity, suppuration, exudation; absence of implant mobility, absence of continuous peri-implant radiolucency, and overall implant survival of 99.5%. Other alloy system used for implant synthesis is cobalt–chromium alloy [56]. Kim and his group used this alloy for fabrication of three-unit fixed dental prosthesis (FDPs) by DMLS process and compared with conventional lost wax (LW) technique. Marginal fit of DMLS was inferior to conventional system but larger than acceptable range [57]. The technology found wider application in the production of various implants like bone analogs [58], orthopedic [59], and dental implants with porous characteristics for dental crowns, bridges, and partial denture frameworks [60–62].

7.7 Challenges and future of 3D printing in dentistry

3D printing technology provided affirmative manufacturing possibilities by the use of wider choice of materials (like polymer, ceramic, metal, and composite) with improved production speed, predictability, low/no waste, accuracy, reliability, and most importantly eliminating the expensive and highly skilled manpower, which is associated in traditional manufacturing practices. It can be used for the simultaneous production of multiple complex 3D materials depending upon the machine's

capacity. For comparison, 50 dental crowns can be produced in a day by 3D printer, which usually takes a considerable amount of time if produced by lost wax casting method. There are substantial applications of 3D printing that bring comprehensive change to the dentistry profession. An article published in the British Dental Journal emphasizes that "The congruence of scanning, visualization, computer aided designing, milling and 3D printing technologies, along with the profession's innate curiosity and creativity, makes this an exceptionally exciting time to be in dentistry." A revolution is underway in 3D printing of dental restorations with the reality of manufacturing of temporary crowns and bridges produced by 3D printing. However, the key challenges stay behind before permanent teeth can be printed. An important attention is also required while multiplying and popularizing the additive manufacturing technology because an upward trend of "do-it-yourself" is monitored in recent few years due to the availability of digital libraries. This could lead the production of unauthenticated materials bypassing the standard testing practices and thus endanger the future and societal faith on 3D printing. Additionally, as discussed earlier, the chair-side tooth digitization is developed and metallic dental restoration is in clinical practice, but patients having metallic implants may not be able to undergo MRI or related type medical screening, thus limiting its adoptability. Therefore, use of appropriate materials for bio-functional tissue-engineered dental restoration is needed to be embrace by additive manufacturing process that can potentially lead dentistry research at an advanced application stage. However, the progressive trend of 3D printing in dentistry is expected to eliminate or markedly decrease the all conventional porcelain metal and ceramic restoration-based approaches. Collectively, additive manufacturing is being constantly progressing and it seems to have a more innovative and advanced future for dental restoration in the years to come.

7.8 Conclusion

Dental implants are a viable solution to replace missing teeth without the need to destroy neighboring healthy dentition. Digital technology and 3D printing have significantly elevated the rate of success and transformed the workflow and practice of dental implant standards of care. The increased production with highest efficiency encourages the use of digital platforms for facing increased economic crisis for rapid production of products in the dental industry. Computer-assisted manufacturing of medical products saves time, increases the productivity, and enhances the health care management of the nation [63]. 3D printing of dental implants favors the additive production to meet increased demands. Although 3D printers are becoming more affordable, the cost of running, materials, maintenance, and the need for skilled operators must also be carefully considered. In spite of these concerns, 3D printing has an increasingly important role to play in dentistry. It is very clear that the coming era surely welcomes digital dental products, which will revolutionize dentistry to achieve more ambitious goals for effective patient care and cure.

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Organ bioprinting

8

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Chapter outline

8.1 Introduction

Every year number of patient dying waiting for transplantation is increasing and the odds of finding perfect match is mostly bleak. It is found that almost 8000–10,000 patients die yearly in United States while waiting for organ transplants [1,2]. The number of patients presently on waiting list in United States is approximately around 120,000 and in most populated countries like China, the number is way above a million [3,4]. This number is set to increase as world ages and arrays of disease that found its way into our system resulting in malfunction of various organs. Only way the demand can be met is if there is custom-made inexpensive technique by which failing organs can be instantly be repaired or replaced [5–7]. This could be possible by the advances made by scientist in the field of tissue engineering. Over last decade, several advances have been made repairing or restoring diseased organs by combining functional cells along with natural and synthetic biomaterials mixed with growth factors engineered to tissue-like architectures [8–10]. While using techniques like particulate leaching or solvent casting [11,12], it was possible to create a cell-laden construct; the major challenge was to control cell distribution in a 3D construct and localize heterogeneity

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of multiple cell type, the intrinsic structures of construct, gradient growth factor distribution, selective augmentation of targeted cells, and induction of blood capillaries [13–15]. To overcome the challenges listed above, an innovative approach of "bio-patterning, bio-printing, and bio-fabrication" was proposed [16]. In this 3D printing type of bio-fabrication techniques since it debuted in 1980s has made tremendous progress and can print few important body parts [17]. Today, it has become one of the most promising and important research areas in tissue engineering and in industrial sector, big boom has led to new era of additive digital manufacturing and additive manufacturing [18,19]. From regenerative medicine point of view, this technique has shown potential to allow millimeter- to centimeter-sized construct fabrication possible and ability to generate complex organ design with ease that was not possible using traditional tissue engineering-based techniques (Fig. 8.1) [20].

The printed bone implants are already seen the light of the day and soon other body parts can also be printed. One of the major players is company called Organovo, that has bio-inks with living cells used for creating liver that can used for drug and chemical testing [21]. Another Russian group has announced printing and testing thyroid gland in living mice [22]. Last 2 years, the company has been offering kidney and soon might be working on printing patches of other tissue. Recently, patient-specific

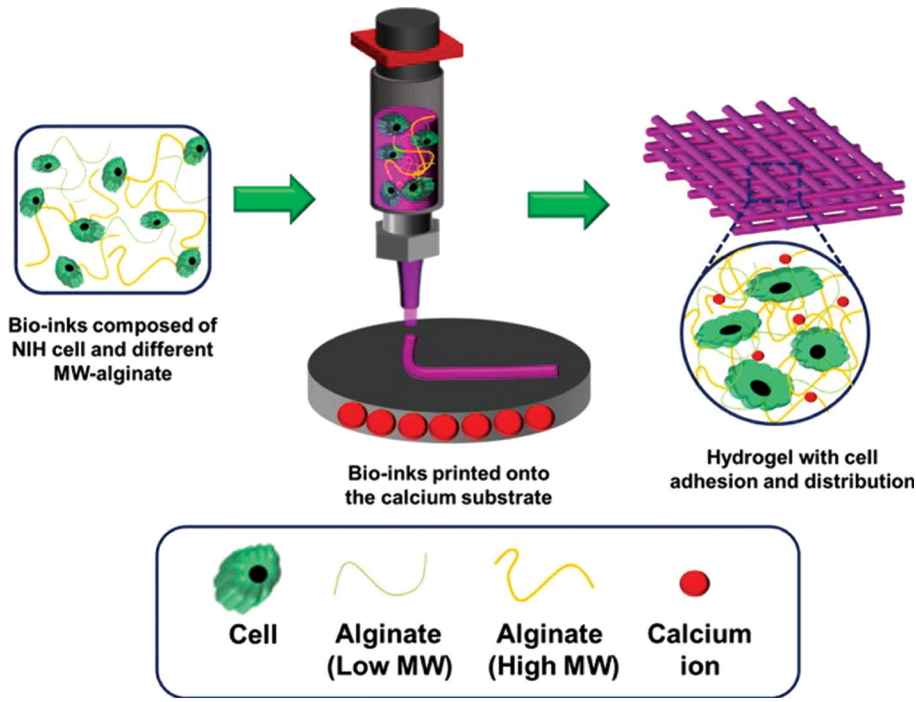

Figure 8.1 Schematic diagram of the three-dimensional (3D) bioprinting process using different bioinks of different compositions.
Source: Reprinted with permission from Ref. [20].

3D printed models of organs or tissue like ear have been used as tool for understanding and exploring anatomical details of patient organs preoperative [23]; this, if successful, can allow surgeons to plan the surgery increasing the successful surgical outcomes. However, making most of the organs achieve the functionality that mimics the natural organ is major challenge.

8.1.1 Present therapeutic intervention for organ failure

The intrinsic purpose of tissue engineering approach is to create a therapeutic substitute that regenerates diseases tissue and organs [24]. When organ functionality is lost due to trauma, age, or congenital defects, various critical and chronic symptoms appear to decrease the quality of life and sometime can be fatal [25–31]. It can be acute or chronic and can remain within one organ or spread across the system resulting in the multiorgan dysfunction. The first choice of treatment is drug; however, this is effective only toward the starting phase. When intensive drug therapy fails, only option is organ transplantation [32]. Waiting for the organ donor can sometime become fatal; hence, best option is replacement of affected organ with artificial one. During last decade, material engineering along with biologist have been able to develop organ and use for augmenting or replicating organ-specific function [33–35]. However, these artificial systems cannot match the biological functionality of the native organ. Consequently, there is urgent need to develop bio-artificial organ that possesses critical, biological, metabolic, and biochemical functions like hormone production, energy generation, and growth factor secretion along with providing immunity [36–39]. Groundbreaking work done by tissue engineers showed the possibility to engineer and produce tissues and organs that in certain aspect can match with the native tissue but none so far has been able to generate fully functional organs. The advancement in the field showed paradigm shift in technique of engineering biomaterials and its bio-functionalities that are enhanced to generate new cell/tissue culture system. But it also played a role in large extent toward development and implementation of the bio fabrication technique also known as bioprinting. To further understand the necessity of advancement, we need to glance through the shortcomings of the traditional tissue engineering and regenerative medicine techniques [40–45].

One of the most widely used regenerative medicine approaches is using cell transplantation but main issue with this approach is difficulty in cell retaining at site of injection. Most of the times, injected cells mostly disappear or remain as bleb causing unwanted immune reaction from the host [46–50]. To prevent cell death during and after injections, hydrogels are most often used as carriers. Hydrogels are altered to gel at body temperature; hence, cells can be mixed with polymer solution and injected into a sol that turns into gel once inside the body. The cells can be at higher density and multiple cells can be injected. Though this is a great technique for in vivo experiments, culturing cells on thermo-sensitive sol–gel can be extremely difficult [51,52]. Another approach is to use 3D scaffold made from either natural or synthetic polymer. In this cell are seeded onto the scaffold, allowed to attach, proliferate and sometime differentiate within this 3D matrix. This construct is subsequently transplanted [53,54]. However, these approaches can be employed only during early

stages of disease where the construct would be able to integrate allowing cells within to immigrate into the host system and perform the functionality. This entire process is time-consuming and in case of critical organ failure, it might not be ideal to deploy this technique since it would give immediate effect as desired in critical cases. It is known that generation and maturation process of important structure must be done immediately following transplantation and it can take longer to duplicate or substitute specific organ functions [55]. This maturation process directly depends upon the cell interaction with the host and there is no control over this process; hence, it is much favorable to implant a fully matured and well functional artificial organ than wait for immature transplant to integrate, develop ,and mature to perform the function in vivo [56]. Therefore, it is critical to absorb the developments of conventional tissue engineering approach and combine the sophisticated technique that can mimic intrinsic details of organ and be readily available for the transplantation and 3D printing perfectly fits the bill in this scenario [57,58].

8.1.2 Factors that influence 3D printing

8.1.2.1 Materials properties

3D bioprinting implements biomaterials to construct extracellular matrix, which can provide conducive environment for cell attachment, proliferation, growth, and differentiation [59]. In vitro microenvironment should be mimicking the in vivo conditions and supporting the cell metabolism. Biomaterials should be biocompatible and biodegradable with appropriate mechanical strength. The scaffold should be able to provide chemical and biological cues to cells and support neo-tissue formation [60–62]. For 3D printing, materials should be able to switch between liquid and solid since it is advantageous to have materials in liquid state during printing and solid immediately post printing for successive deposition and complete model layer by layer [63]. Most of the time, this technique requires cross-linking methods as an additional step. Along with all these characteristics, materials should have appropriate degradation rate and ability, which means rate of its degradation should match up with the rate of neo tissue generation and its ability to break down into byproducts that can pass through renal and blood threshold without having any impact on the local cells and other parts of the body. This phenomenon is also known as cytotoxicity free materials [64].

8.1.2.2 Printing precision

For 3D printing, the best state is to control single cell deposition; this allows cells to be accurately placed in the construct to form an ideal organized structure [65–67]. This is especially good for multicellular organs in which each cell has distinct functionality, and single-cell control can simulate the body structure to large extent and should be exactly at same organizational position as native tissue [68,69]. In such case, the interaction between adjacent cells can be controlled artificially. As bioprinting techniques develop, single-cell deposition onto 2D or 3D environments has been widely used to explore cell behavior and to monitor response to physical, chemical, metabolite, and cytokine stimulation [70]. Significant progress has been made to ensure minimal

cell damage and maximum cellular comfort by mimicking the extracellular environment of the tissue. In case of organ printing using these stem cells, these cells must differentiate in controlled manner to produce cell types of desired linage by placing them in specific location within 3D construct and can result in functioning neo-tissue formation [71]. For example, islet cells with secretory function account for ~2% of pancreatic cells. Printing these functional cells and introducing them in patients with disease pancreas would still allow the tissue to produce insulin and aid in restoring the functionality tissue [72].

8.1.2.3 Environmental control

External factors such as temperature, humidity, and gaseous concentration also play critical role in maintaining the activity of tissues and organs. Temperature is known to have direct effect on the activity of cellular protein in 3D scaffolds, which has great influence on the neo-tissue formation and function. Similarly, humidity seems to have effect on the cellular growth and proliferation too. Different research groups working on the 3D printing have found that ideal temperature for biological samples seems to be between 29 and 31, especially for collagenous tissue also, relative humidity should remain between 65% and 85% [73]. However, these are not strict and numbers can vary depending upon the cell types. Furthermore, in case the starting materials are stem cell or pluripotent stem cells, it would be ideal to start with 20% CO_2 than the usual 5% as it would allow better proliferation of stem cells; however, for differentiating stem cells, gas concentration can vary and each cell type needs to be optimized as there is no ideal number that would work in all conditions [74].

8.1.2.4 Aseptic conditions

As requirement for precision printing increase so would the printing time and that in turn increase cell exposure to the outside environment [75]. This would mean higher chance of contamination possibility and to maintain aseptic condition takes precedence. Aseptic processing is required to be carried out gradually according to printing process and machine status to ensure highest cell viability of organ's printing. Higher cell viability in the printed organ would allow the tissue to survive longer and with full functionality [76].

8.1.3 Benefits of 3D printing of organ

8.1.3.1 Custom-made personalized construct

One of the biggest advantages that 3D printer offers in the regenerative medicine is the freedom to custom-made medical supplies [77]. For example, using 3D printer, one can customize prosthetics and other implants that provide greater value for both patients and surgeons. Furthermore, 3D printing can also produce made-to-order fixtures for operating room usage. These made-to-order fixtures and implants can have positive impact in terms of the time needed for surgery, recovery time, and success outcome of the procedure as the tissue is printed to be closer to the patient's own

tissue type. It is also expected that 3D printing technique will eventually allow to analyze drug dosage forms and release profiles administrated to be customized for every patient [78].

8.1.3.2 Enhanced productivity

In comparison to the traditional tissue engineering and regenerative medicine, 3D printing can be "fast," which means any entire tissue or organ can be printed within few hours [79]. This makes 3D printing more attractive process; also, since almost entire protocol is computerized, the variability and errors are minimized. Medical implants and prosthetics that are made have better resolution, reliability, accuracy, and repeatability than tissue generated using traditional tissue engineering approach, and it might be difficult to achieve this kind of precision with other 3D engineering techniques [80].Fig. 8.2

8.1.3.3 Increased cost-efficiency

There is another advantage using 3D printing that is ability to reproduce tissue replica at cheaper rate. Using other manufacturing techniques remains less expensive when done in large scale; however, the cost is competitive when done in small scale. In case of small size implants like spine or craniofacial, the cost to print these implants is as expensive as it would be to grow these tissues in the lab using traditional tissue engineering-based approaches [82,83]. Also, in the companies that print complex

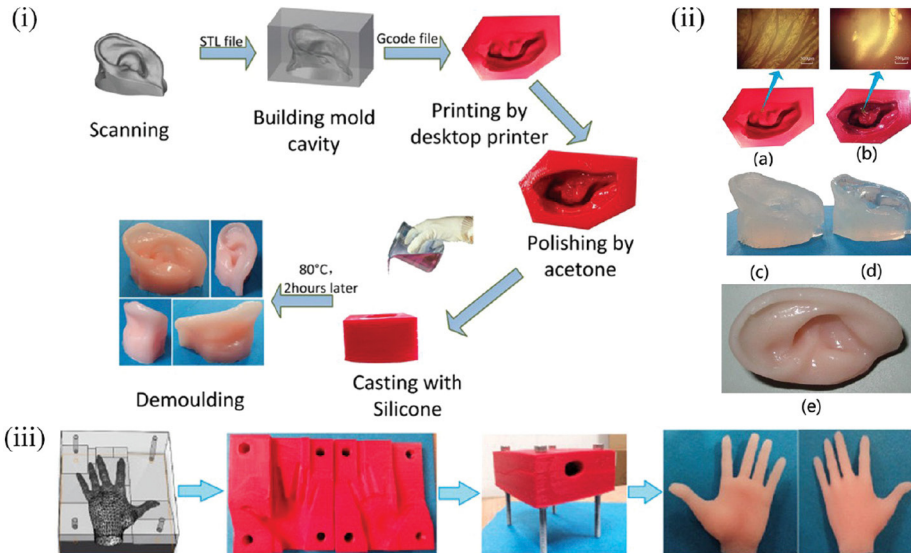

Figure 8.2 Steps involved in typical scanning printing polishing casting and used for printing ears (a-unpolished, b-polished, c- casted with unpolished and d-ear casted with polished mold, finally e-fine silicone ear). Similar technique for engineering hand prosthesis. *Source*: Reproduced under open access from Ref. [81].

organs that require frequent updates or produced in low volumes, it works out cheaper to adapt the changes in the 3D printing set up than other engineering techniques. 3D printing can reduce the production cost by decreasing the unwanted resources. Since the 3D printer allows manipulation and alteration in micron level, it would be much cost-effective to feed the numbers into the scanner and allow it printed the altered 3D tissue, whereas other tissue engineering techniques do not offer the flexibility to alter at micron level and thereby in the end, the neo-tissue might be of the quality that is required for transplantation. Cost adds up in every step from optimization to final product and 3D printing technique is catching up with the others in terms of being cost-effective by increasing the efficiency of the products printed [84,85].

8.1.3.4 Democratization and collaboration

Biggest advantage of 3D printing is that it aims to make the implants and prosthetics easily accessible to every patient. As there is growing demand for organ transplant, it become impetus to have cost-effective, easy available and patient specific organs. An increase range of materials is becoming available for 3D printing the cost of printing organ decreases, thereby allowing more people both from science and medical field to utilize to play around with design and produce novel design that can ultimately provide relief to patients suffering from organ malfunctions. The nature of 3D printing is such that it needs team of researchers and doctors and it gives unprecedented opportunity for sharing knowledge and data [86]. Unlike most of the other fields of research, 3D printing team can download the .stl file from open source and produce exact replica of medical device or model letting accurate sharing of designs. In 2014, NIH established 3D printed database that allows sharing 3D print files for anatomical models, custom labware, medical, and replicas of proteins (Fig. 8.3) [87].

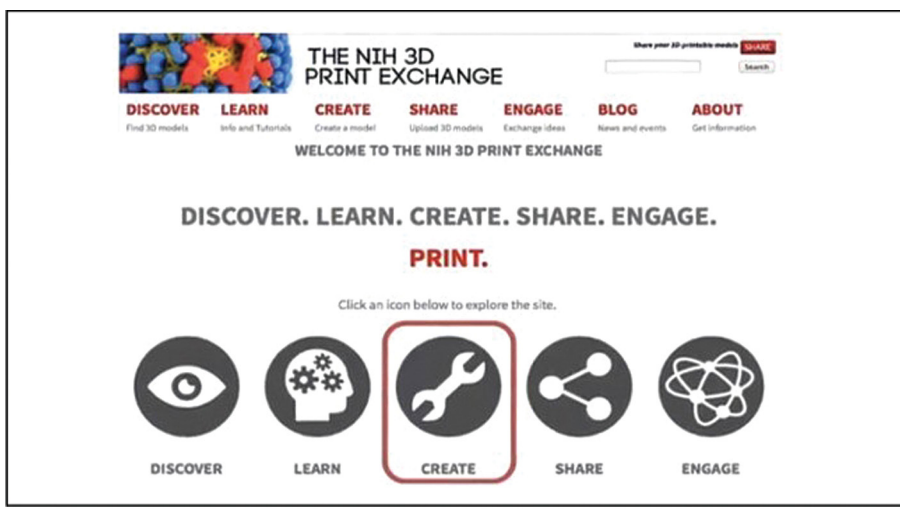

Figure 8.3 NIH central open source website that allows 3D printer researchers and medical to free share 3D print files and tutorials [87].

8.2 Bioinks design and techniques for organ printing

3D printing allows precise and predesigned geometry, composition, and structure to overcome the bottleneck of 2D culture and other traditional tissue engineering scaffold-based technology. This allows spatial manipulation of cell and biomaterials knowns as "Bioinks." [88] This allows study of cellular interaction within 3D construct, influence of biomaterial on cells, and formation of functional tissue and organs. Bioinks can be used in printing if they satisfy certain materials and biological criteria [89]. As a material, it should be printable, degradable, functional, and should have certain mechanics. For biological aspect, main requirements would be biocompatible, nontoxic, and bioactive. For a material to be accepted as printable, it needs to comprise of two important parts: (1) ease of processing it into bioink formulation and (2) the print fidelity is usually associated with the mechanical strength of the final construct printed to self-sustaining 3D structure. Depending on the printing protocol, it could involve bioink viscosity, cross-linking characteristics, and surface tension properties [90–92]. Of this, viscosity is crucial as it has direct impact on print fidelity and cell encapsulation efficiency (Fig. 8.4) [93]. Polymer with high viscosity is less likely to flow freely during printing process, thereby affecting the mechanical integrity of the printed structure. Furthermore, it might need high pressure for flowing through limiting the gauze size and printing of minute details impossible.

The most widely used bioinks for tissue/organ printing are cell-laden hydrogels [94,95], tissue strands [96], microcarriers [97], spheroids [98], cell pellet [99], and solutions based on decellularized extracellular matrix [100,101]. Among this, cell-laden hydrogel is extremely attractive due to its tunable characteristics and its capacity to recapitulate the cellular niche. Similarly, the decellularization of native tissue is emerging field and has found wide acceptance due to its inherent bioactivity and ease

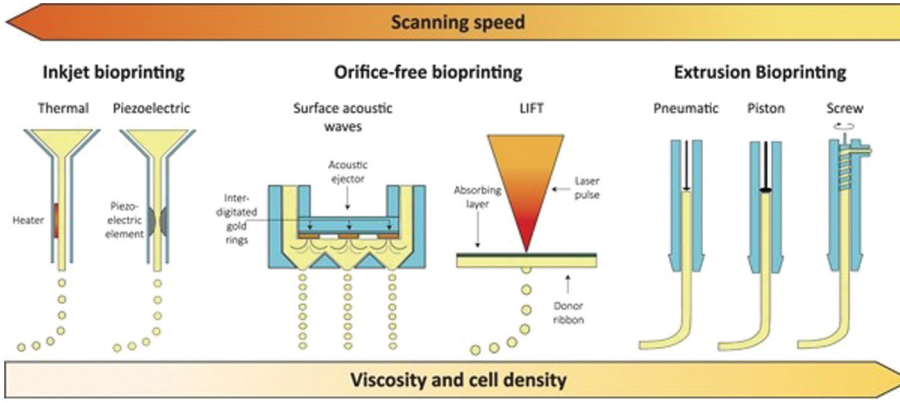

Figure 8.4 Overview of the most widespread bioprinting approaches and according parameters crucial for printability of the material from bioink properties before, during and after 3D bioprinting.
Source: Reprinted under creative commons license from Ref. [93].

with which it can be made into bioink for printing. Lastly, cell suspension inks are usually based on cell aggregates that act as biological construct that are scaffold-free.

8.2.1 Cell-laden hydrogels

Compared to the traditional scaffold based techniques, cell printing overcomes the limitation of low cell delivery efficiency along with uncontrolled biomaterial-cells distribution by controlling deposition of cell-laden. This is most often used bioinks as it can be made easily for process called extrusion-based like drop in water, droplet based such as inkjet and laser based like SLA and LIFT (Fig. 8.5) [102].

This bioink formulation uses natural polymers such as alginate, chitosan, agarose, college, hyaluronic acid, gelatin, and fibrin as well as synthetic materials like poly (ethylene glycol) and pluronic (poloxamer) or blends of both [93]. Advantage of using natural material is it offers inherent bioactive site that interacts better with cells except agarose and alginate, which are considered neutral; rest of the polymers are structurally like native ECM. For example, collagen polymer chain is linear and filamentous structure displays the strain–stress behavior of a soft tissue in the body. Most of the natural polymers have active polymer chain that can be functionalized with various chemical moieties to induce cross-linking or enhance bioactivity [103]. On contrary, synthetic materials do not promote or enhance cellular functionality naturally but can be functionalized to provide cues to achieve better cellular response. However,

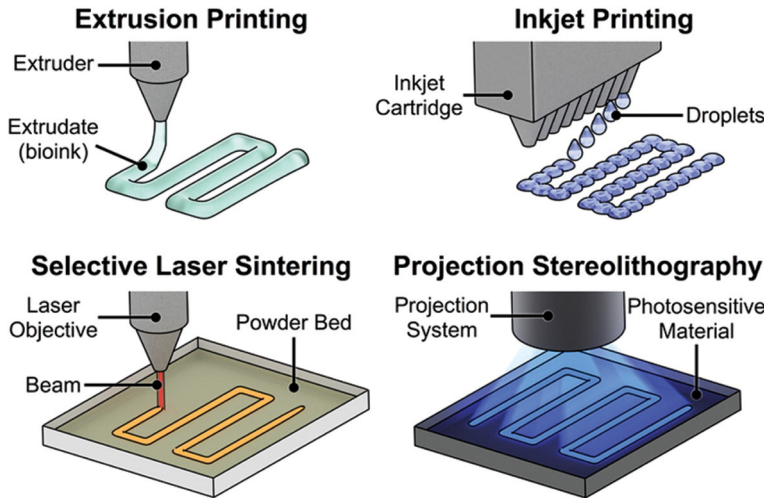

Figure 8.5 3D printing methods commonly adapted for biomaterial fabrication. Extrusion printing and inkjet printing rely on liquid intermediates or precursors, which can solidify quickly after ejection. Selective laser sintering provides localized heating to melt or fuse powder granules. Stereolithography relies on photo-induced polymerization of a liquid resin in the specific regions exposed to light (artwork by Jacob Albritton and Jordan Miller). *Source*: Reprinted with permission from Ref. [102]. Copyright © 2016 American Chemical Society.

in comparison to the natural polymers, synthetic hydrogels have tunable mechanical properties. By blending natural and synthetic polymers, one can achieve tunable biological and mechanical properties and by incorporating nanoparticles, bioink formulation can be optimized for different application [104]. Commonly, all hydrogel bioink preparation requires printing of polymer solution followed by cross-linking step [105]. This needs highly viscous polymer solution with weight percentage of polymer around 3% and rapid cross-linking after printing to develop self-sustaining structure. There are two forms such as physical and chemical cross-linking in which physical is nonchemical-based approach that uses hydrophobic, ionic, and hydrogen bonding interactions between the polymer chains. Chemical cross-linking relies on the covalent interactions like click chemistry [106], enzymatic reaction [107] or Michael-type addition reactions [108], and photo-induced polymerization [104]. Hydrogel cross-linking or gelation is an important aspect of preserving shape of bio-printed structure, thereby minimizing collapsing of construct [109]. When the 3D construct in chemical cross-linked, it had mechanical strength and stability that is far better than the physical gels and this could be important factor that affects the cell–material interactions and can exert influence over the stem cell behavior during differentiation [110,111]. There are different examples of cell-laden hydrogel techniques in which polymers like Pluronic and PEG are most commonly used synthetic materials. Pluronic, a poloxamer-based triblock copolymer, is synthesized using two hydrophobic groups between water-soluble sets and has been used in extrusion-based printing since its temperature is sensitive and turns gels at room temperature whereas flows like liquid at 10. However, this is found to be not stable and erodes in short time; hence, it is usually used as a supporting material [112]. Chang et al. applied different approach in which alginate in pre-crosslinked condition was explored and alginate/gelatin-blended inks were used using extrusion-based bioprinting techniques, and it was found that gelatin increased printability [113]. In another experiment, Nair et al. used viable endothelial cells during bioprinting and it was found that dispensing pressure played critical role in cell viability than diameter of the nozzle [114]. Furthermore, Hendriks et al. engineered model to connect rate fibroblast viability with cell containing droplet size, substrate properties, and velocity of printing in droplet-based deposition system [115]. Works done by Catros et al. determined correlation between thicker substrate, low laser energy, higher velocity of bioinks, and cell survival in laser-assisted printing [116]. In another study, 3D printing of HEK293FT cells with gelatin-based hydrogel found that nozzle insulation greatly increased cell viability after printing [95]. All these studies focused on influence of bioink's properties and different printing parameters that must be tuned to ensure good printability and high cell survival. Otherwise, either the cell-laden hydrogel would collapse, lose accuracy and or alter structural integrity during longer culture duration, or worst cells might undergo apoptosis and/or experience a change in the phenotype. More recently, Zhao et al. demonstrated that viscoelastic properties of bioinks in decisive factor for both print fidelity and cancer cell viability when all other parameters like printing speed and extrusion flux are maintained constant. It was also mentioned that different cells might require different viscoelastic range [25]. Work done by Blaeser et al. shows that shear stress can also be key factor against which to balance cell integrity and printing resolution in a

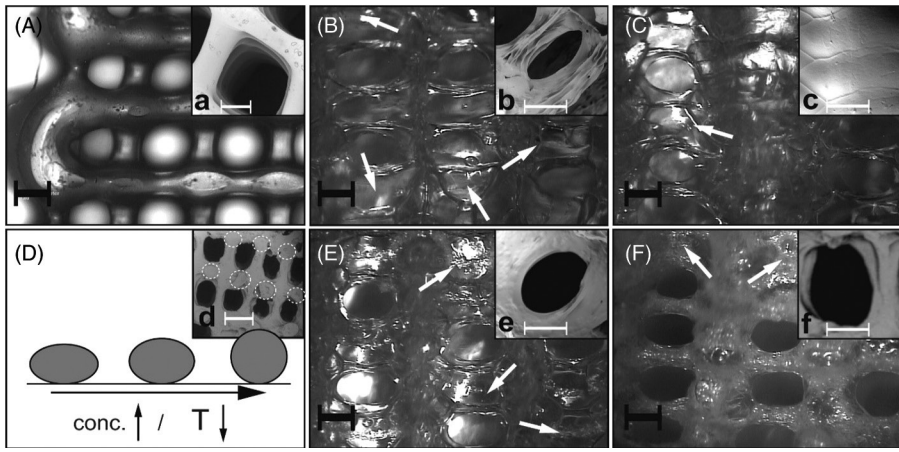

Figure 8.6 Scaffold pore architecture is influenced by the hydrogel concentration. Light microscopy and SEM images representing 10% w/v scaffold pore geometry in top view (A, a), cross-section (B, b), and side view (C, c). Cross-section images of 15% w/v (E, e) and 20% w/v (F, f) scaffolds. White arrows indicate partial collapse of the subsequent layers. Scale bars indicate 200 µm. Schematic representation of the deposited strand geometry as a function of initial concentration and plotting temperature (D), accompanied by a cross-section SEM image indicating the strut geometry of 20% w/v scaffolds (d). Scale bar of (d) indicates 400 µm. *Source*: Reproduced with permission from Ref. [73]. © Elsevier 2014.

value-based jet printing technique. It was found that shear stress should be within 5 kPa to obtain 90% cell viability [117]. Billet et al. showed influence of hydrogel concentration, the printing temperature, pressure, speed, and cell density and found that 100% interconnected porous network could be fabricated with gelatin concentration of 10–20% w/v (Fig. 8.6) [73]. While fabrication of cell-laden scaffold encapsulated with hepato-carcinoma cells, it was identified that printing pressure and shapes of the needle impacted cell viability. Using all these feedback strategies, one can combine chemistry, engineering, and print cell-laden hydrogel that can protect cells and delivery with high efficiency than conventional techniques.

8.2.2 Bioinks in extrusion bioprinting

Perhaps the most commonly used fabrication technique of 3D cell-laden construct is via extrusion bioprinting. Extrusion-based bioprinting (EBB) has grown substantially in the last decade. It is versatile in printing cells, tissue constructs, tissue, organ modules, and microfluidics devices, in applications from both basic and clinical research [118]. The pressure or extrusion method techniques have been used for long time in plastic and metal molding and shaping. However, in the late 90s with the emergence of fused-deposition modeling (FDM), extrusion-based techniques showed that it was possible to print 3D construct with intricate geometries and controlled porous architecture. This technique was later introduced in tissue engineering and many pioneering

works done demonstrated in literature includes developing printable biomaterials and scaffold fabrication by Hutmacher's group [119,120]. Also, exploring different design and modeling aspects for solid free foam fabrication of scaffolds by Hollister's group has shown impact of this technique in regenerative medicine [121–125]. There are wide variety of the bioinks such as hydrogels as mentioned early, micro-carriers, spheroids, tissue strands and decellularized components of ECM. This wide range is due to the nozzle diameter that is used for EBB, the ability to deposit small building blocks in fugitive liquid medium, ability to extrude bioinks in almost solid state, and flexibility in nozzle tip design. Different bioinks applicable in EBB are alginate, chitosan, gelatin, poly (ethylene oxide), methylcellulose, Matrigel, and fibrin to name few.

Alginate is biocompatible, low cost, and fast gelating polymer that has been widely used in EBB. Different EBB systems have been experimented with to manipulate instant gelation of alginate by using ionic calcium, calcium carbonate, sulfate, and chlorides. These mechanisms are bioplotting, bioprinting hydrogel with secondary or co-axial nozzle using cross-linker deposition and spraying system, bioprinting of pre-crosslinked alginate that is further processed for crosslinking and bioprinting using aerosol cross-linking process (Fig. 8.7A) [126].

There is often misunderstanding in using bioprinting and bioplotting; however, bioplotting technique uses hydrogel solution extruded into a plotting media usually cross-linker pools, extrusion takes place within this pool and bio printed scaffold stays inside the pool until the completion of the process. Hence, extrusion of hydrogel without cross-linker plotting media does not qualify in the “bioplotting” approach [127,128]. In bioplotting, the density of the bioink to be extruded must be greater than the plotting medium for successful deposition process. In second approach (Fig. 8.7B2), the cross-linking solution is sprayed or deposited onto the printed alginate using secondary nozzle that rotates around the primary nozzle with help of motorized system. In the third technique (Fig. 8.7B3), alginate is printed using coaxial nozzle system through the core and cross-linking solution is ejected through the sheath section of outer nozzle that is slightly longer than the core nozzle that provides better control on the extrudability of bioinks. Using similar technique but an opposite configuration in which co-axial nozzle development, alginate bioink is extruded for various applications like bioprinting of blood vessels, creating multi-materials fibers for controlled drug delivery and microfluidics channels used in tissue engineering. Fourth technique (Fig. 8.7B4), pre-crosslinked alginate is bioprinted, providing an adequate deposition quality of bioink and structural integrity of scaffold, followed by increased crosslinking by exposing the scaffold to high concentration of cross-linking solution. In this approach, better mechanical strength is achieved but the pressure level is relatively higher to pre-crosslinked hydrogels. Furthermore, the bioink is not uniform, which brings discontinuity during extrusion. The fifth approach is alginate and is bioprinted onto stage, where the cross-linker is fumed using ultrasonic humidifier over the entire setup (Fig. 8.7B5) [128–131]. Difference between this and spraying technique is that fuming process generates small particles of cross-linker that is in near vapor state and can be homogenously distributed over the entire bioprinted construct as opposed to spraying technique. This allows simultaneous cross-linking between layers while fabrication generating mechanically and structurally stable constructs. All these approaches have pros and cons but it has shown that bioprinting pre-crosslinked

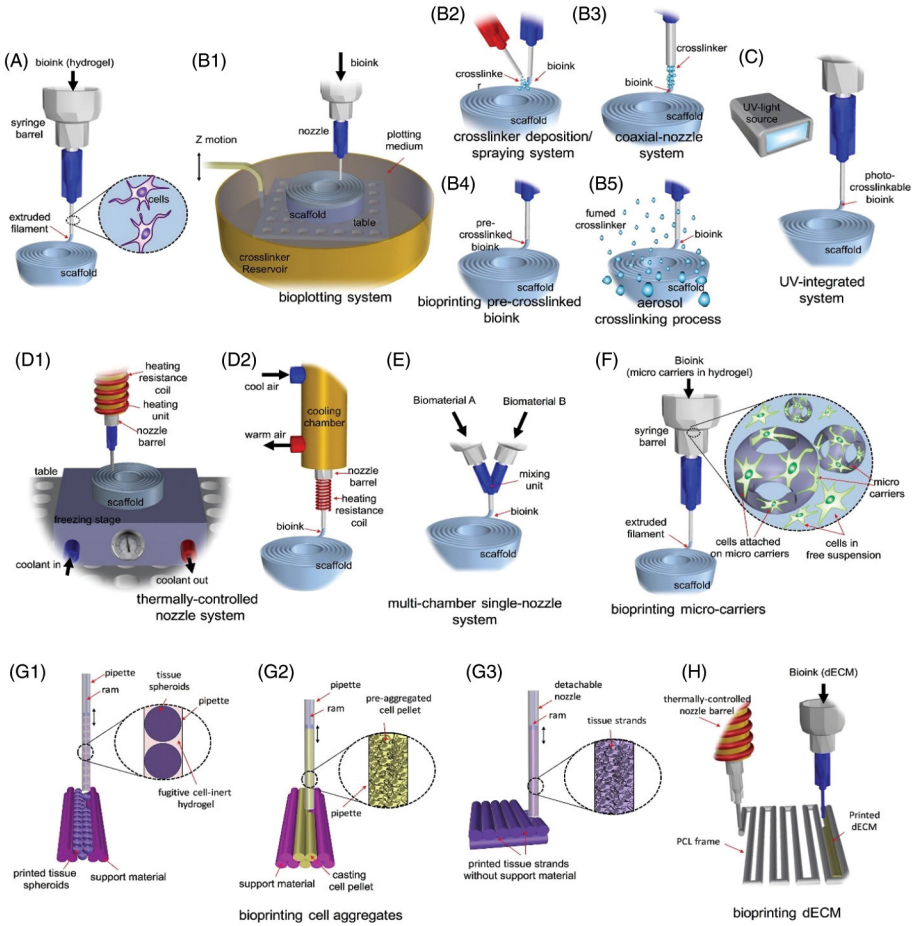

Figure 8.7 Processing configuration for different bioink materials: (A) hydrogel-based bioprinting-based bioinks, (B1) bio-plotting hydrogel bioinks into a reservoir meant for cross-linkers, (B2) spraying system or cross-linker deposition, (B3) coaxial-nozzle system, (B4) bioprinting of pre-crosslinked bioinks, (B5) aerosol cross-linking system, (C) UV-integrated system, (D1) heating unit assisted barrel with cooling unit assisted bioprinting stage, (E) multichamber single nozzle unit, (F) bioprinting microcarrier preloaded with cells as delivery medium that will be extruded in hydrogels, (G1) extrusion of tissue spheroids in a short cell inert (inert to cell adhesion) hydrogel into a support material for fusion and maturation of the spheroids, (G2) bioprinting of preaggregated cell pellet into a cell inert support material, (G3) bioprinting of tissue strand directly without using support mold or medium, and (H) bioprinting of dECM within a printed PCL frame to provide mechanical support to gelation of dECM. *Source*: Reprinted with permission from Ref. [126].

alginate bioink shows promising results for postprinting construct stability, printing accuracy, and ability of the construct to integrate well with interlayers [132–136].

Gelatin is hydrolytic derivative of collagen, with high water adsorbing capacity, good biocompatibility, and nonimmunogenicity, that completely degrades in vivo

[137]. This is thermos-reversible hydrogel that is solid in low temperature and solution at 37; however, it has low mechanical strength and usually unstable in physiological conditions. For using in EBB, different chemical and physical cues such as glutaraldehyde or metal ions are used as cross-linkers to improve bio-printability and stability of the hydrogel. For synthesizing gel that can be stable at 37, photopolymerization is done by chemically modifying methacrylamide side groups [39,73]. This reaction takes place in the presence of water-soluble photoinitiator. The resulting bioinks can easily be extruded through pneumatic dispenser equipped with UV [15] (Fig. 8.7C).

Hyaluronic acid (HA) is a natural nonsulfated glycosaminoglycan modified with methacrylate like gelatin to manipulate its photopolymerization (Fig. 8.7C). HA-MA bioinks can be used for EBB with high printable capacity [112,138]. It is extensively used as filler by dermatologist and lubricant in synovial joints for arthritis treatment.

Agarose **is** a galactose-based polymer that can be thermosensitive and thermo-reversible hydrogel. There are different varieties of the polymer available in market and depending upon the hydroxyethylation, the melting temperature can vary [139]. The most suited agarose for EBB is one with low melting and gelation temperature. Since it is already shown that agarose is biocompatible and can support cell differentiation, for EBB application (Fig. 8.7D1), agarose can be used to bioprint at low temperature and extruded agarose bioink solidifies rapidly when printed onto the freezing stage. Campos et al. have shown that the bioinks used for printing tubular structure supported cells and have 100% viability even after 3 weeks indicating feasibility of using this as bioinks for printing 3D constructs [140].

Pluronic is a triblock copolymer based on poly(ethylene-glycol)-block with poly (propylene glycol) and poly (ethylene-glycol) sequence that has been approved by FDA and widely used as drug carrier and as injectable gel for treating burns. The intermolecular association of PPO is attributed to its temperature-sensitive property as it leads to formation of micelle above the critical micelle temperature. Example 20% of pluronic-F127 would gel above 20°C and this sol–gel temperature can be modified by changing the solution concentration and this presents unique opportunity to be used in EBB process [111,113]. A thermally controlled nozzle system that can solidify bioink during extrusion process is needed (Fig. 8.7D2). When the bioink is loaded onto the barrel in liquid state, the temperature is kept lower than the room temperature and heating unit near the dispensing tips gives precise control of temperature during bioink's extrusion [141]. This way, bioink can be extruded in solid filament shape and forms. However, it is very unstable and mechanically weak polymer and researchers are contemplating using this as sacrificial material or a fugitive ink to engineer vascular networks [111].

Fibrin is extensively used in tissue engineering due to excellent cell-adhesion capabilities and high cell seeding density [142]. It is simple ionic reaction that gels this polymer in the presence of Ca^{2+} along with fibrinogen and thrombin at room temperature. Its polymerization condition can be manipulated depending upon the cell types or stiffness range by altering the concentration of the solution. Though it is difficult polymer to process there are ways in which it could be used for EBB. First, both fibrinogen and thrombin individually are excellent for printing and theoretically can be extruded [143]. Second is to mix both these components on ice to prevent early gelation and then extrude using specific configuration (Fig. 8.7D2). Third technique

is using multichamber, single nozzle system that mixes both fibrinogen and thrombin into one solution at the very end of the process (Fig. 8.7E) [144]. This approach has been used for blending and printing multiple hydrogels or for making same hydrogel with different material properties to obtain heterogeneity in extruded fibers. Using these bioinks, different hydrogels with varying properties can be bioprinted; however, hydrogel generally lacks suitable biomimicry for bioprinted cell phenotypes. While most of the hydrogels used does not contain all the proteins required for cell growth and loading high cell density ranges closer to natural tissue can be challenging. It is generally considered that higher cell density better cell interacts with each other and in turn forms better tissue. Cell interactions can be affected by the limitation of no of cells that can be bioprinted using bioinks, thereby reducing efficient cell interactions. Although hydrogel bioprinting seems feasible, biggest challenge is degradation of the construct and its associated byproducts along with mechanical stability. In general, hydrogel degrades very slowly and can be identified in some case in the in vivo even after 5 weeks causing concerns about use of hydrogel-based bioinks [20,143,145].

8.2.3 Microcarriers

Another recent advance has been made in using reinforcement blocks in EBB process. In this, cells are loaded into small carriers of different shape with porous architecture (Fig. 8.8) [146]. Commercially available carriers for cartilage and bone regeneration are made from dextran, glass, plastic, collagen, and gelatin. When cells are cultured on these constructs, they allow quick cell proliferation [147]. Matured microcarriers are printed in delivery medium like hydrogels (Fig. 8.7F) and similar cross-linking step is used. It is found that cell–cell interaction is better inside microcarrier than cells in a hydrogel solution [148]. These have great potential in the scale-up tissue printing process in which hard polymers are used. In general, hard polymers are not feasible for encapsulating cells due to their limited diffusion; however, microcarriers can be made porous and loading cells on such porous structure allow them to proliferate and generate carriers in 3D hard tissue scaffolding application especially for cartilage and bones [149]. As attractive as these microcarriers are, there is still a need to optimize different parameters like how to assemble them into a 3D construct, how to successfully print them on bioprinting stage, and how to have microcarriers interact with each other to form stable system. Other concern is degradation process of these microcarriers and its byproducts can be toxic to the cells that need to be addressed before using microcarriers as potential bioinks in bioprinting [150].

8.2.4 Cell suspension bioinks

Modified inkjet printer has for long time been used for printing cells into cellular assemblies. For example, endothelial cells were printed from suspension into growth media by Wilson et al. Bioprinting of scaffold-free 3D constructs uses cell aggregates in form of either mono or multicellular spheroids as bioinks. This bioink undergoes total biological self-assembly process without presence of any temporary support layer as it relies on tissue liquidity and fusion process that allows cells to self-assemble and fuse together due to cell–cell interactions. One of the examples is the work done by

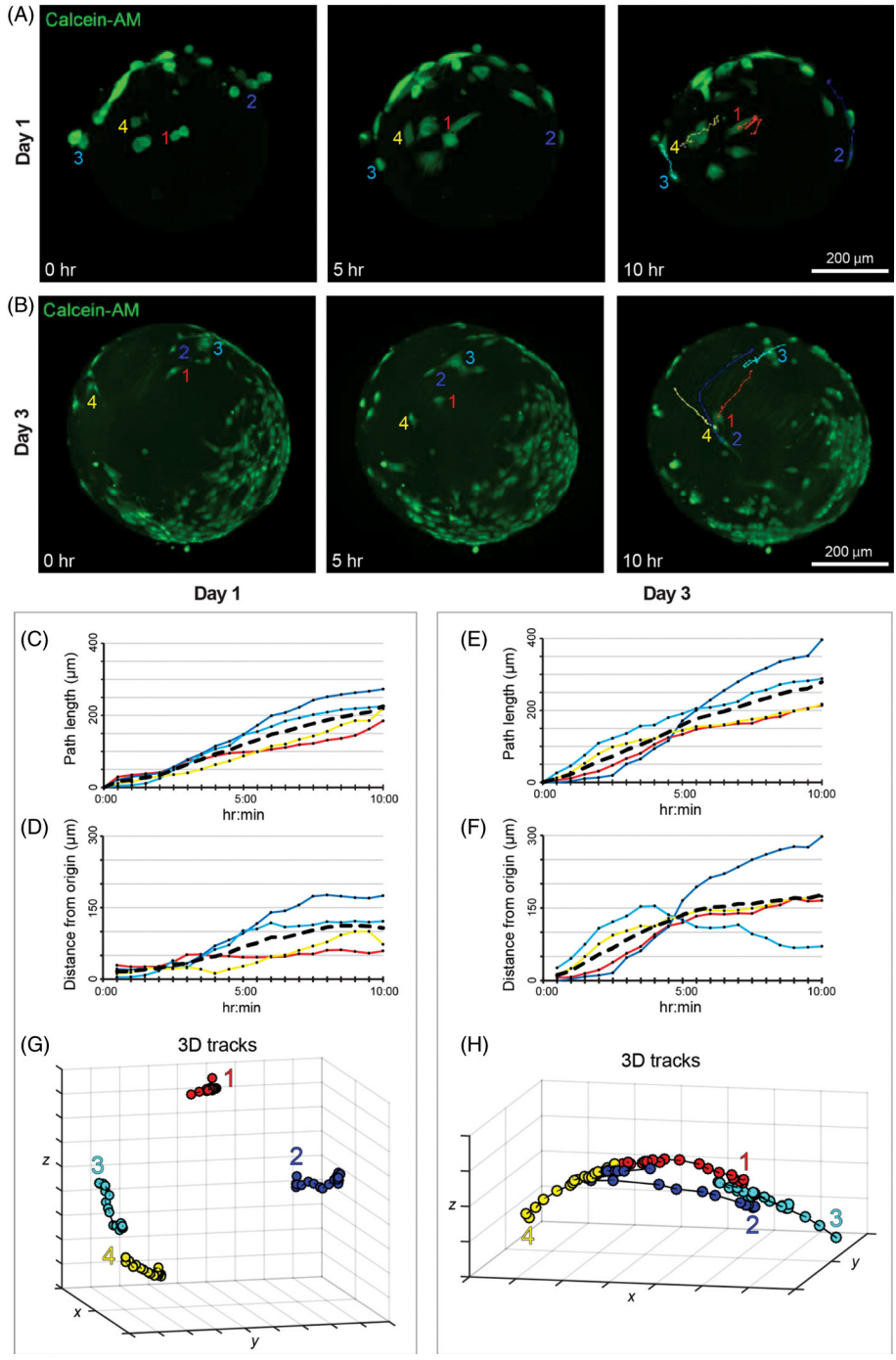

Figure 8.8 Representative frames of the evaluation of motility of MSCs loaded onto the microcarrier at day 1 and day 3 of culturing after the initial loading. Single green channel showing Calcein-AM positive cells is shown for three different time points (0 hr, 5 hr, and 10 hr) on Day 1 and 3 (A, B).

Norotte et al., whose group developed spheroids and cylindrical multicellular aggregates with controlled diameter of 300–500 μm range and show that postprinting fusion leads to single- and double-layered vascular tube formation [151]. One of the biggest companies in bioprinting, Organovo, uses similar approach to engineer functional human tissue toward in vitro disease models. They have developed liver models using high-density bioinks obtained from parenchymal cells and nonparenchymal cells that are printed using EBB. Tissue can mature in a bioreactor that mimics native tissue environment for 3 days to form scaffold-free tissue. In 2014, Levato et al. developed alternative technique that combined cell-laden PLA-based microcarriers for extensive expansion of cells. This scaffold-free construct allows quick maturation of building blocks and has been used for developing cardiac patches, nerve tissue, and blood vessel [97]. This technique also includes hanging drop, micromolded (nonadhesive) hydrogel, pellet (reaggregates), and conical tube culture. Despite being promising approach it has its drawbacks for EBB use like loading tissue spheroids into a nozzle system is difficult (Fig. 8.7G1) and it requires delivering medium for extrusion. In that case, a fugitive ink or thermo-responsive gel is used that is inert to cells. Ehsan et al. fabricated vascularized tumor spheroids and used it for studying early stages of tumor progression, and this was great example of how cell aggregates can be vascularized and in vitro replica of tissue model and can be used for creating other organoids [152]. From bioprinting prospective, printing cell aggregates is very trivial when cell pellet is loaded onto nozzle system in a preaggregated form (Fig. 8.7G2) while bioink can be printed as hydrogel-based bioinks without any other support. The mold structure must be printed without or minimum cavity or else cell pellets would not form the aggregates and remain in suspension [153]. However, for the scale-up process, this dependency of mold is not ideal and when it is printed on the mature tissue, spheroids, or strands, then printing is no longer trivial and the bioinks should be transferred to the stage in usually solid form with minimum stress to cells. Major obstacle in this approach is that mold itself starts to support the tissue growth and maturation and cells might not use the mold matrix for proliferation and it becomes major hurdle in large-scale production and sometimes tissue strands can be used as supportive material (Fig. 8.7G3) [154]. Alternatively, hydrogel or biological oil can be used as delivery medium and can be washed away in postprinting process. In general, cell aggregates must be printed before they reach maturation stage such as before day 10 or else they might lose their fusion property. However, despite difficulty in processing these bioinks, they are extremely valuable technique and one of the best hopes rapidly creating tissues in the in vitro condition that is structurally, mechanically, chemically, and biologically functional to qualify for organ transplantation.

8.2.5 Decellularized matrix components

Besides all the advances in hydrogel-free techniques, using the extracellular matrix made for patient's own tissue as scaffold is considered as a new bioink that can be source for advanced tissue engineering. The groundbreaking work done by Taylor et al. in organ decellularization has attracted immense attention and in last 5–8 years has enabled work on regeneration of liver, heart, kidney, and pancreas [155]. Drawing inspiration from this work, Dong-Woo and group used decellularized tissue component in printing tissue analogue. They decellularized and chopped tissue into smaller fragments, which

were then used for loading cells and printed onto PCL frame that acts as support material (Fig. 8.7H) [156]. Three different cell types have been tested for these constructs and it has successfully demonstrated to achieve natural differentiation of cells when it is loaded in their native dECM. This approach seems to have potential to be next big technique; however, there are limitations like low availability of the organ, affordability of bioink, and scale-up process. Since the native tissue is decellularized and chopped into smaller fragments, a large volume of original tissue would be required for the scale-up process. Furthermore, certain toxic residues can still stay in the process dECM. Due to these challenges, printed bioink cannot enable cell formation while cells can be absorbed into the matrix components or the matrix shrinks significantly. (Fig. 8.9)

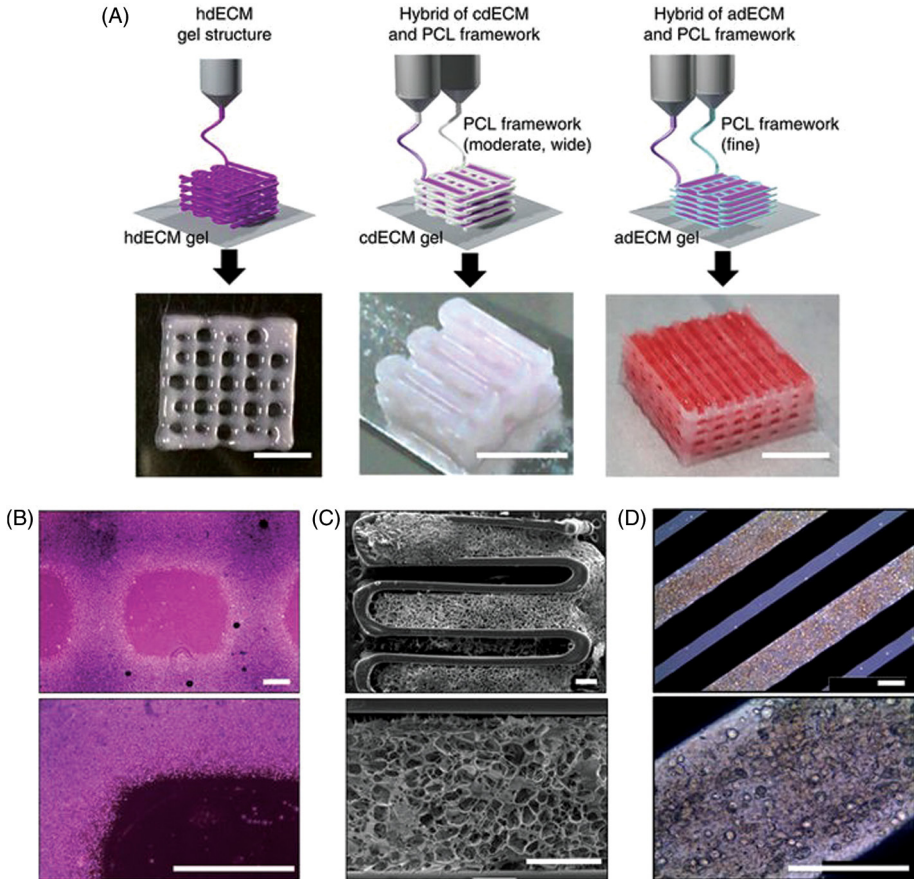

Figure 8.9 Heart tissue construct was printed with only heart dECM (hdECM). Cartilage and adipose tissues were printed with cartilage dECM (cdECM) and adipose dECM (adECM), respectively, and in combination with PCL framework (scale bar, 5 mm). (B) Representative microscopic images of hdECM construct (scale bar, 400 μm), (C) s.e.m. images of hybrid structure of cdECM with PCL framework (scale bar, 400 μm) and (D) microscopic images of cell-printed structure of adECM with PCL framework (scale bar, 400 μm).
Source: Reproduced with permission from Ref. [156].

8.3 Bioprinting of organs

Challenge for tissue engineering is fabricating a 3D vascularized cellular construct of clinical relevance matching the shape, size, and structural integrity of natural tissue. Bioprinting has shown promise for generating composite constructs by precise placement of cells in hydrogel in layering fashion. There are different techniques employed to achieve this goal like laser-induced forward transfer (LIFT), extrusion, and jetting techniques. By using these different technologies, 3D construct can be generated ranging micrometer range and can be used for delivering viable cells, macromolecules, and biomaterials that can generate 3D tissue constructs.

8.3.1 Vascular system

Even though creating vascular features in printed tissue is usually limited, novel methodology can help overcome this limitation in which coaxial nozzle system is used to print vascular conduits that can be a meter long. In this, carbon nanotubes reinforced alginate conduits that were perfused to support the growth of coronary artery smooth muscle cells cultured within the matrix. Using coaxial nozzle system, this group could engineer conduits with diameters in submillimeter range; however, the possibility of printing close to capillary diameter seems difficult. Another approach that can be explored using magnetic controlled nanoparticles is bioink for printing vessels. In this, position of vessels can be controlled by applying magnetic field. Further research is needed to determine the efficiency and potential of this approach. Another technique is using sacrificial inks for printing vascular channels and different bioinks have been used for this like Pluronic F127 that prints channels as small as 45 μm and were later endothelialize it with HUVECs [157]. This technique combines printed fibroblasts encapsulated within gelatin methacrylate bioink that yields multicellular bioprinted structure. After printing, the temperature is lowered to allow Pluronic F127 to melt exposing the open vascular channels that is ready to be seeded. Another researcher group previously used carbohydrate glass as sacrificial materials in various bulk ECMs to form channels that could be used for seeding cells and where as small as 150 μm (Fig. 8.10) [158]. To use the lattices as sacrificial elements in creating channels in a monolithic cellularized tissue construct (Fig. 8.10A). There tactics was to used suspension of cells in ECM premade polymer is casted to the encapsulated lattice. After crosslinking the ECM, the carbohydrate glass in dissolved to form vessels while its interfilaments fuse to form intervessel junctions (Fig. 8.10B and C). To show the flexibility of this approach, Miller et al. [158] patterned vascular channels with live cells in different types of ECM (natural and synthetic) materials (Fig. 8.10D). The time needed for ECM prepolymer and cross-linking along with glass dissolution is in order of minutes and can quickly encapsulate cells. Most important aspect of this study was to choose different ECM materials that varied in its size and cross-linking property. This technique generated channels without requiring further modification. As expected from optical transparency characterization of carbohydrate glass, photopolymerized gels demonstrated no visible shadowing artifacts and no other technique (ionic, enzymatic, or protein precipitation) can be used for wide variety of ECM materials to create channels with this kind of optical transparency. This methodology

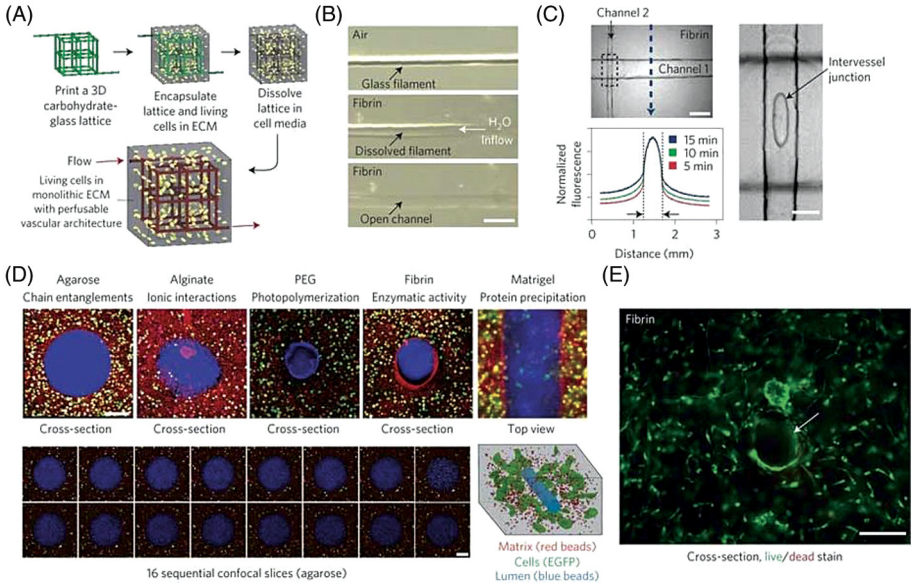

Figure 8.10 Monolith tissue system containing patterned vascular structure along with living cells (A) schematic overview showing open, interconnected, self-supporting sacrificial carbohydrate glass lattice for casting vascular architectures. This lattice is within ECM along with live cells. This can dissolve within few minutes in cell medium without affecting nearby cells. The process results in monolithic tissue with vascularized architecture that matches the original lattice. (B) A single filament measure about 200 μm is diameter is encapsulated with fibrin gel. After cross-linking, the gel and filament are submerged in water that dissolves carbohydrates resulting in channels and removing of filament leads to open perfusable channels within the gel. (C) Fibrin gels are patterned interconnected with channels of different diameter supports, diffusive and convective transportation of fluorescent dextran injected into the channel network. (E) Cell seeded onto these constructs expresses EGFP in wide range of ECM materials. (E) Cross-section of perfused construct after 2 days shows high no live cells indicating the convective flow within the system were supportive for cellular proliferation and viability. *Source*: Reproduced with permission from Ref. [158].

has no adverse effect on the cells and encapsulated cells were found to be viable, proliferating and migrating into the channels of scaffolds at level like nonchannel matrix demonstrating compatibility of entire vessel casting process (Fig. 8.10E). Although this approach demonstrates the potential of this approach in supporting modified cell lines, primary parenchymal cells that can be used for the transplantation were found to be susceptible to the stress of hypoxia and suspension. Nevertheless, this study proved that sacrificial carbohydrate glass lattices are suited for creating densely populated tissue system with perfusable vascular channels and junctions. Biggest advantage is the fact that entire system in perfusable within minute and continuous phase simply fills 3D void volume that is around the carbohydrate glass lattices.

8.3.2 Human Mandible bone bioprinting

The bone and cartilage tissue are interesting in both traditional and bioprinting techniques as it poses to influence the filed [159]. This can be custom-made and use anatomic data obtained from patient CT scans. Initially, rabbits were used for scanning and testing polycaprolactone-hydroxyapatite scaffolds that could support the relevant loads. Wang et al. [160] used poly (propylene fumarate) to print porous scaffolds and characterized its degradation rate for over 224 days and demonstrated that the printed scaffold was stable to be used for bone tissue engineering. Another work done by Pati et al. showed osteogenic potential of 3D printed PLGA/PLA/β-TCP matrix by using human mesenchymal stem cells derived from nasal inferior turbinate tissue for depositing bone like ECM. After short culture period, scaffolds were decellularized and tested both in vitro and in vivo in which it showed to improve osteo-inductive and conductive property [161]. However, work done by Kang et al. [162] established that cell-laden hydrogel along with synthetic biodegradable polymer does have the mechanical strength and structural integrity, and is vascularized to be used for tissue engineering. They printed human scale mandible bone and evaluated its characterization and function in the in vitro and in vivo. The human-sized mandible structure was engineered in size and shape that is needed for reconstruction surgery after traumatic injury (Fig. 8.11). The cells used in this experiment were human amniotic fluid-derived stem cells that can differentiate into the osteogenic linage with right growth factors. Mandible bone has uninformed shape, and CT scan data of mandible defect with Mimics software was used to produce CAD model of the defect shape (Fig. 8.11A). A text-based command program generates CAD model with customized CAM software and determines the required dispensing paths of cell-laden hydrogel from mixture of PCL, Pluronic F127, and tricalcium phosphate (Fig. 8.11B). PCL/TCP along with hAFSCs mixed gels is printed on type 1 pattern with Pluronic F127 acting as temporary support (Fig. 8.11C). 24 h post seeding cell viability was found around 91 $\pm$ 2% confirming that printing process does not have negative effect on cell viability. After osteogenic induction at day 28, the bone was stained using alizarin red to confirm calcium deposition in control (Fig. 8.11D) and the cell-laden construct (Fig. 8.11E). Using ITOP, authors could engineer 3D free-form shapes with different cells and materials leading to various architectures that potentially can form vascularized tissue types. In this study, it was demonstrated that it was possible to achieve consistent reproducibility of tissue with complex architecture that can be vascularized and suitable for clinical applications.

8.3.3 Neuronal tissue

The peripheral nervous system is known for limited innate capacity for regeneration following any trauma or surgery. For injuries larger than few millimeters, autografting is normal standard practice although this procedure has considerable donor site morbidity and is limited in its availability. Due to this, nerve guide conduits (NGCs) are promising alternatives but mostly have limited efficacy for small or large injury gaps in comparison to the autografts. Bioprinting nervous tissue is most widely researched

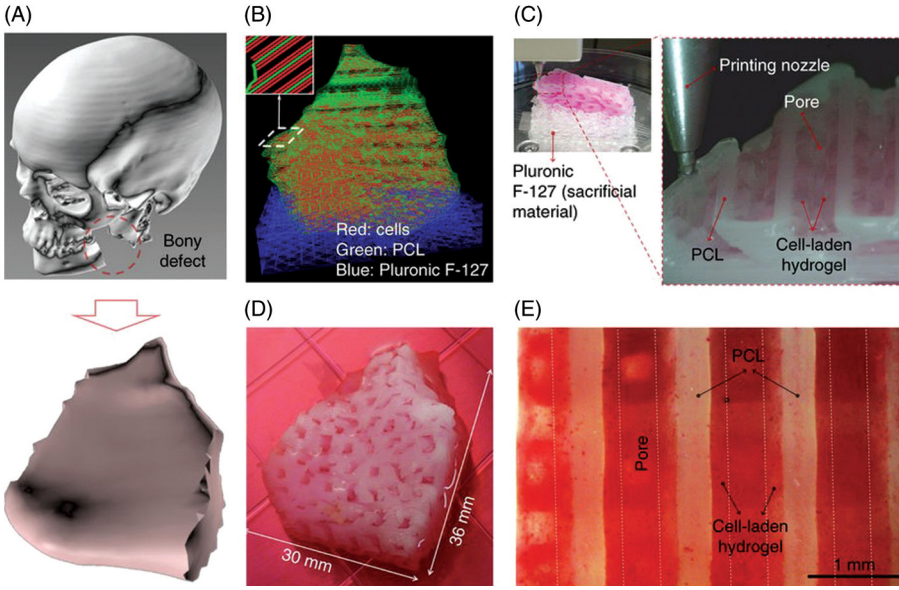

Figure 8.11 (A) Three-dimensional CAD model identifies mandible defect from human CT scan data. (B) Visualized motion program coded to construct a 3D architecture of mandible bone defect by using CAM software developed at Atala's lab. Line of different color indicates the dispensing path of bioinks. (C) 3D printing techniques using integrated organ printing system showing patterning of layer of the printed construct. (D) Image of 3D printed mandible bone defect seeded with cells for osteogenic differentiation for 28 days. (E) Osteogenic differentiation of the cells confirmed by alizarin red staining for calcium deposition. *Source*: Reproduced with permission from Ref. [162].

among bioprinting researchers. A large synthetic tissue that integrates with nervous system and bioprinting may be how new nervous tissue can be engineered, or enhance the innervation of construct [99]. Different groups have engineered synthetic nerve grafts using cells alone in which isolated bone marrow-derived stem cells and Schwann cells were casted into 500 µm diameter tubes and loaded onto bioprinter that extruded discrete tubes for dense nerve conduit of Schwann cell tubes that is surrounded by bMSCs for animal application [163,164]. This is an early-stage proof-of-principle graft that performed as good as control tissue and is promising technique that required further exploration. Another experiment performed by Lorber et al. [165] provided added validation on the potential of using printing cells for nervous system by using retinal ganglion cells and glia cell mix in inkjet printing systems. Peteman et al. [166] used micro-stereolithography technique for designing NSGs for peripheral nerve using poly(ethylene glycol) of low molecular weight with photocurable property. Neuronal cell cultured on photocured PEG and TCP showed that cell growth in PCG was comparable to that of control. For evaluation of lithography construct before in vivo implantation, ganglion cells derived from rat dorsal root were used. For this purpose, hemisphere "trench" were engineered of 2 mm dia half-circular channels

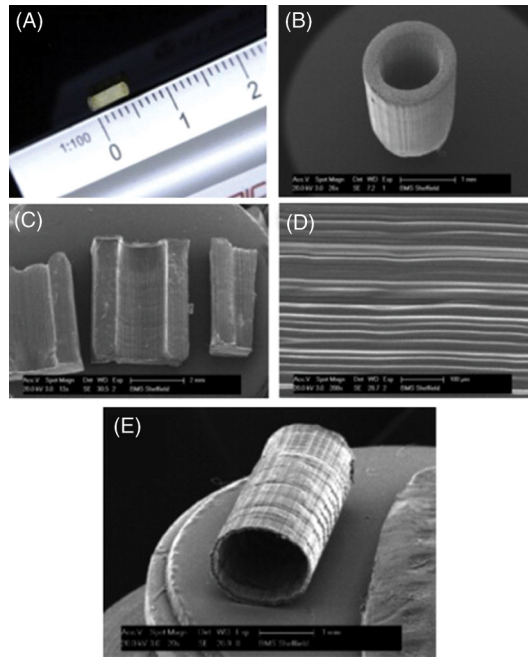

Figure 8.12 Optical and scanning electron microscopy image of typically PEG NGC for 5 mm l × 1.5 mm diameter with wall thickness of 250 µm. (C and D) SEM image of trenches at different magnification. (E) An experiment PEG NGC mage with wall thickness of 50 µm to demonstrate the resolution that can be achieved using micro-stereolithography.
Source: Reproduced with permission from Ref. [166].

within 2 mm height, 3.5 mm width and 10 mm long rectangular block of PEG. This was done so that the bottom of the well has some curvature of 1mm internal dia tube (Fig. 8.12). This can be used easily in 6-well culture plates with limiting movement of the conduits (Fig. 8.12) and can be reproduced. The system can be used to achieve low resolution of about 50 µm and can be upscaled. While PEG is not typically supportive to cells, once it is photocured, it was found to have better cell attachment. Overall, this study demonstrated the feasibility of using stereolithography for rapid and precise production of NGC that has intrinsic property and can be customized.

8.4 Future and concluding remark

In the future, bioprinting is going to be intrinsic part of artificial organ generation. Due to its many advantages like printing organ in millimeter scale, precision, and highly controlled dispensing of live cells, it will play vital role in regenerative medicine. Bioprinting can be applied in deposition or encapsulation of live cells in desired place and position. Bio-sensor, protein, and DNA array already use bioprint-

ing techniques and this diversity shows the versatility of its usage even though this approach is still considered to be in its infancy. Moreover, it remains a promising solution to organ shortage, transfer of infection or antigen, or morbidity at donor site. Its ability to generate tissue for transplantation on demand that is custom-made reduces the risk of transferring the antigens or infection from donor organ; also, it does overcome immune rejection issue. Recent progress made in hydrogel technology like dynamic switchable gel or oxygen-producing gels allows research to have better control over the microenvironment for cells. As technology matures, bioprinting is positioned to be key approach in fabrication of human-on-chip systems along with on demand anatomically.

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Biomanufacturing

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Chapter outline

9.1 Introduction

Tissue engineering is a multidisciplinary field that combines principles of biology, chemistry, materials science, mathematics, engineering, and clinician toward development of live functional biological substitutes that restore, repairs, or regenerate the lost functionality of the tissue [1,2]. It was initially defined as *application of principles and methods of engineering and life sciences towards the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and development of biological substitutes to restore, maintain or improve tissue function* by Skalak and Fox in 1988 [3, 4]. Advances made by scientist have made the treatment for diseases like Alzheimer's [5], Parkinson's [6], and spine injuries [7] possible in near future by aiming to regenerate or repair the damaged tissue [8, 9]. The technique of achieving this is based on interplay between three critical components, (1) Scaffold [10], (2) cells, and (3) growth factors, of which scaffold plays vital role as it provides structural support for the cells to proliferate along with mechanical stability for neo-tissue regeneration and chemical cues for cells to differentiate into the desired linage. The idea is to seed the cells into the scaffold, and interaction between the cell and the scaffold would trigger the differentiation pathway in the cells and induce the cells to produce its own ECM that turns into a functional tissue [11]. It also allows better nutrient transportation in a 3D system along with exchange of waste materials and gases. Hence, scaffold is termed as critical in both ex vivo and in vivo as it serves the above-mentioned purpose along with acting as cell carrier that absorbs the stress during the transplantation and keeps higher

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keeps higher number of cells alive, which is a desirable aspect for every live graft. Furthermore, scaffold could stay at the site of transplantation till the cells migrate out and interact with the host cells leading to better integration of graft with the host tissue. There are three main approaches in tissue engineering that involve: [12]

1. Cell-based approach, which involves direct implantation of the cultured cells or cell substitutes in the in vivo system and it is based on the principle that once in the body with conducive environment, cells would be able to synthesize its own matrix. Tissues are usually built from different cell types arranged in specific format and alignments in 3D or 2Ds making its own functional matrix [13,14].
2. Growth factor-based approach, which involves growth factors that are usually signaling molecules that regulate a multitude of cellular functions like differentiation, proliferation, migration, adhesion, and gene expression. Based on the type of cells, different growth factors can be incorporated and tuned to have controlled release to prolong the effect of the molecule to cells [15].
3. Scaffold-based approach, which involves fabricating 3D material, which act as a temporary substrate for the cells to attach, proliferate, and differentiate on these structures that gives not only physical support but also mechanical and chemical cues for formation of new tissue. In this strategy, implanted cells attach to the scaffold, grow, secrete its own ECM, and stimulate neo-tissue formation [16].

Whichever approach used by bioengineer scaffold plays impetus role in regeneration of the tissue and strongly depends upon the materials and its manufacturing processes. Scaffold materials includes natural and synthetic polymer along with ceramics, metals, and composites. Each type of polymer provided different characteristics to the scaffold such as natural polymer is known to be favorable for increased cell attraction and attachment than synthetic and metallic polymers; however, material made pure natural polymers mostly lacks mechanical strength to support growing cells; hence, finding ideal combination for types of tissue needed for regeneration is important. Similarly, there are many different techniques for manufacturing scaffold using freeze-thawing to electrospinning and newly emerging 3D printing.

9.1.1 Conventional techniques scaffold engineering

Solvent casting/salt leaching involves combining solid impurities like sieved salt particles with polymer solution and then casting the dispersion to produce a membrane that has both polymer and salt [17,18]. In the final step, salt is leached out of the membrane using water yielding a porous membrane. This is most straightforward method in which the particulate is leached out and although it is used for fabricating cylindrical structure, the users have no control over the pore size and shape. Irregular shaped pores without interconnectivity are not acceptable for tissue engineering scaffold. Different approaches have been used to overcome this drawback that is due to using of salt or sugar as porogens, like using salt fusion at elevated humidity [19]. This technique while allowing some interconnectivity, still produces irregular shaped pores with large strut thickness. Furthermore, by using this approach, one can synthesize simple scaffold geometry but nothing with complex tubular architecture can be obtained. Another technique that has been utilized to improve the pores architecture and

optimum pore interconnectivity is using spherical porogens like gelatin [20]. Gelatin spheres have produced highly interconnected pores but to completely remove gelatin from the final membrane is proven to be difficult. Recently, researchers have used hydrocarbon spherical porogens stabilized by another commonly used polymer poly (vinyl alcohol) seems to be feasibly way to make highly interconnected porous membrane [20]. These spherical shaped porogens, however, usually result in pore diameters outside the range of the particle size, which could be due to either interparticle or shrinkage or cohesion effect. Major drawback in this approach is lack of control over the pore size, distribution, and interconnectivity of the pores. Furthermore, to recreate the intrinsic architecture of the tissues and organ, this is a least preferred technique since it is almost impossible to attain critical precision that is needed for the task.

Another technique known as gas foaming uses organic solvents at high temperatures [21]. High pressure carbon dioxide is used for fabrication of the scaffold, and pores architecture and size depend upon the amount of the gas mixed in the polymer [22]. This approach involves exposing highly porous polymer mixed with gas at high pressure ~800 psi to saturate the polymer solution with gas. Under these conditions, gas separates from the polymer causing phase separation from the polymer. The CO_2 molecules becomes cluster to minimize the amount of free-energy available that in turn results in pore nucleation. These pores cause significant loss in polymer density and gain in the polymeric volume creating a 3D porous scaffold structure at the end of the process [23,24]. Here again, the porosity of the scaffold is controlled by using different porogens like salt, sugar, and wax. This leads to porous scaffold without interconnected pores that in long term affects convective flow of nutrients [25].

Each of these techniques presents various challenges as it usually does not result in ideal scaffold properties such as control pore size, architecture, and spatial distribution of pores that are not interconnected and restricted internal channels within the scaffold. The shape and size of the pores can be altered by changing parameters of techniques, which results in scaffold formation with random pore distribution, leading to inconsistencies in scaffold architecture that obstructs the supply of nutrients from medium and ingrowth of tissue into the scaffold. Besides, most of these techniques involve usage of solvent that is toxic to cells along with long fabrication times and labor-intensive process. Due to all these reasons and many more biomanufacturing additive fabrication techniques is considered as viable alternatives to designing scaffold especially for tissue engineering and regenerative medicine as it offers better architectural control for scaffold fabrication. This methodology provides ability to design porosity and internal connection within the 3D scaffold as it is more design-dependent opposed to the process-dependent tendency of conventional techniques, which allows wide range of processible materials as well as consistent defined microarchitectures.

9.1.2 Biomanufacturing additive processes

This represents a new group of nonconventional scaffold fabrication techniques introduced recently in the bioengineering field [26,27]. The biggest advantage of this methodology is its capacity to rapidly design and produce complex 3D construct with intrinsic network in layer-by-layer fashion by using wide range of raw materials. It

combines the clinical imaging data that are used as raw data for replacing defect in customized fashion. Some of these processes are performed in room temperature, thereby making it possible to encapsulate cells and protein without altering viability and protein structure. This is critical for tissue engineering and regenerative medicine as this technique can be used for fabricating scaffold with customized shape, architecture, and internal channels with predefined morphology giving much need control to the manufacturer. Most of the common methodology includes stereolithography [28], laser sintering [29], extrusion [30], and layer-by-layer 3D printing [31]. This process of 3D printing was developed by Charles Hull in 1986 by using stereolithography, which was further modified and new techniques like fused deposition modeling (FDM) [32], powder bed fusion and inkjet printing [33], and contour crafting (CC) [34] emerged in late 1990s. From these basic techniques, various new methods, materials, and equipment have evolved in the last decade with the capacity to transform manufacturing and logistics processes. AM has been used widely in various industries like biomechanics [35], prototyping [36], and construction [37]. With everyone year passing, new application for AM has emerged in which novel materials are being developed and one of the main reasons of this development is expiration of original patent that gives manufacturers ability to develop new 3D printing constructs without restriction. Recent development has also resulted in cost reduction; therefore, larger markets such as schools, homes, lab, and libraries are able to utilize AM for various projects. Initially, 3D printing was mostly used by architects, but the cost was astronomical for producing custom-tailored products for end users. However, with all the modification and new raw materials, 3D printing small quantities of customized goods has become relatively low cost. More recently, China has effectively used 3D printing to mass produce cheap houses (4800 USD) [38] printed in 24 h by WinSun. However, the biggest impact of AM is in the biomedical field as it allows unique patient-specific products' development in relatively lower cost and lesser time frame. Customized functional products have much needed requirement and have become trend in treating patient who otherwise would be depending on organ donation and transplantation procedures. This methodology has gained much attention in biomedical due to its ability to produce variety of implants using CT-scanned image of tissue replicas [39,40]. AM allows fabrication of parts in different sizes ranging from micro- to macroscale. However, depending upon the accuracy of measurement and detailing of the raw data dictates the outcome quality of the finished product. For achieving success in microscale, it is impetus to have high-resolution raw data yet there is challenge to attain surface finish and layer bonding that usually needs postprocessing modification such as sintering before the end product. Despite all complexity distinguished advantage of 3D printing is its ability to mass customization, that is, manufacturing of series of customized goods that can be different at low cost as the mold or tool for printing does not require change due to the change in the dimension of finished product. All changes can be fed to the software that allows the cost-effective production of personalized goods. Printing complex construct at fine resolution is the reason AM was developed, and rapid prototyping can achieve this in printing of large structures that reduces printing defect and increase mechanical properties are some of the reasons that driven the expansion of AM techniques. The most common methods in 3D printing use polymer

filament known as fused deposition modeling, along with using powders by selective laser sintering (SLS) and laser melting (SLM) [40] or using liquid binding in 3D printing (3DP) in addition inkjet printing, stereolithography (SLA), contour crafting, and direct energy deposition (DED).

9.2 Inks: 3D printable biomaterials

Tissue-engineered and 3D printed structures require a biomaterial that mimics the chemical composition, the mechanical properties, and the structural support of the ECM. These printable biomaterials must be biocompatible to minimize and/or avoid any inflammatory response. Natural cellular ECM production is one of the end goals these biomaterials should enhance. Medical devices and tissue engineering are one of the most important fields where 3D printing is becoming the most significant techniques. AM has the potential of improve patient-specific needs; it reduces the costs and the time frames to create the needs of the patients. AM is the secret for personalized medicine soon and thus a wide range of biomaterials are being used and studied as bioinks. A wide range of chemical composition, strength, stiffness, and size have been used as shown in Fig. 9.1.

However, most biomaterials and bioinks started to be widely used in industry; therefore, they lack biocompatibility and in certain cases, biodegradability [45,46]. These two aspects are at the front of tissue engineering and regenerative medicine. For 3D printing to compete in the biomedical treatment, studies must focus on integrating biocompatibility in their bioinks: synthetic polymers have tunable properties and

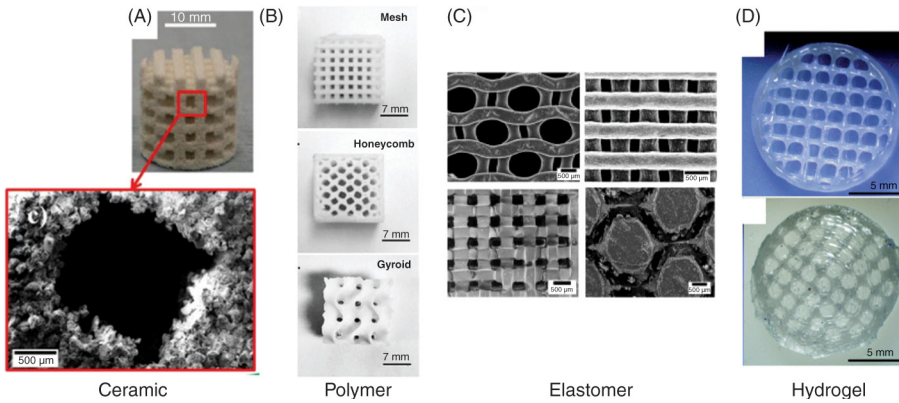

Figure 9.1 3D printed biomaterials in nature. (A) (top) 50 wt.% hydroxyapatite (HA) scaffold, (bottom) SEM image of the ceramic particles. Reproduced with permission from Ref. [41]. Copyright 2015 Elsevier. (B) Polycaprolactone scaffold. Reproduced under creatives common attribution law of open access from Ref. [42]. (C) Images of 4-layer PDMS lattice printed by sequential depositing. Reproduced under creatives common attribution law of open access from Ref. [43]. (D) pHMGCL–NHS 3D-printed network and pHMGCL–NHS–PNC–PEGNHS thermoplast–hydrogel construct. Reproduced with permission from Ref. [44].

therefore can be biocompatible; natural polymers are by definition biocompatible and most of the time biodegradable; hydrogel can be made of synthetic or natural polymer combined with water.

This section aims at defining and listing most of the different bioinks that have been used in biomanufacturing and tissue engineering. Material properties, mechanical properties, use, and applications are detailed into the different material classifications: polymer, hydrogels, inorganic, and composite scaffolds. Their specific benefits and drawbacks will also be mentioned.

9.2.1 Polymeric scaffolds

Synthetic and natural polymer scaffolds have finely tunable properties. This gives them the ability to be biocompatible and even biodegradable in certain cases. Polymers are, by definition, an addition of a certain number of monomers. This structure is defined on a nanoscale level and the backbone (part of the monomer) can be made of synthetic or natural components. Polymers make up the majority of the biomaterial used in 3D printing due to their low cost, tunable properties, ease of process, and major properties such as biocompatibility, mechanics, and degradation [47,48]. For example, taking the printing design from the original ceramic models, a mixed biodegradable polymer powder has been used by Griffith and others with the SFF method to bind 25 % poly(L-lactide) (L-PLA) and 75% poly(lactide-*co*-glycolide) (PLGA) using chloroform as a binder [49]. The end product of this AM was to create and print a branched liver construct with its internal architecture being as close as possible to the reality [50]. This liver was later improved with cell seeding (hepatocyte and nonparenchymal liver cells) to enhance the liver cell metabolism.

Polymer inks come in many forms, which are used in the different AM techniques: filaments for FDM, gels for DIW, solutions for SLA, powders for SLS. Each of these forms and techniques requires specific characteristics and parameter of the material for printability. Polymer filament must have a diameter of 1.75 mm to be used for FDM techniques, their elastic modulus to melt viscosity ratio has to be below $5 \times 10^5\ s^{-1}$ to prevent filament buckling and clogging of the FDM. Shear thinning problems in liquid form can be countered by a sharp solid-to-melt transition, which allows also to facilitate extrusion [51]. The important factor to take in consideration for gels and solutions for DIW is the organic solvent used to dissolve the polymer. These organic solvents, such as dichloromethane or tetrahydrofuran rapidly evaporate when the extrusion starts and therefore leave only the polymer strut behind. Shape integrity and support must be maintained and thus these gels must dry in seconds to minutes. In addition, solvents must evaporate completely post manufacturing, gels must be shear thinning to avoid clogging and their viscosity has to be low enough to print a low pressure and shear. Powders and beads for SLS require a diameter in the range of 10–150 μm to allow particle to flow in the bed [52, 53]. Low viscosity and high temperature of melt are needed too (around 200°C). Finally, stereolithography relies on photo-cross-linkable polymers that polymerize rapidly when subject to UV radiation. Crosslinking should be as fast as possible to maintain resolution and speed while density and viscosity much be in relation with the final 3D printed product. Based on

these design parameters, many different materials have been fashioned into printable polymer bioinks for 3D printing technologies.

9.2.1.1 *Poly (lactic acid)*

Polylactic acid (PLA) is the most used polymer for FDM due to its low cost, nontoxicity, biocompatibility, biodegradability (to a certain extent), and easy processability [60–62]. PLA melting temperature is around 175°C, and extruding temperature being usually around 200 and 230°C PLA can be formed into filaments for use with melt-based printing systems. PLA structure can be seen in Fig. 9.2. Biocompatibility and biodegradability are one serious concern surrounding PLA. Indeed, PLA degrades via a reaction called hydrolysis: this reaction releases acidic byproducts, which can compromise long-term biocompatibility. These acid byproducts [63,64] could lead to tissue inflammation and cell death. This hydrolysis reaction corresponds to the degradation of the ester bond and results in the localized decrease in physiological pH through the release of lactic acid. In order to create composite scaffolds with increased bone response and reduce formation of a localized acidic environment, PLA has been combined with ceramics and calcium phosphates [65,66]. Composites of PLA with ceramics can also be beneficial to modify and increase the compressive strength and improving the elasticity of the implanted material (increasing Young's modulus). PLA has good mechanical properties for a synthetic polymer but tends to be brittle and

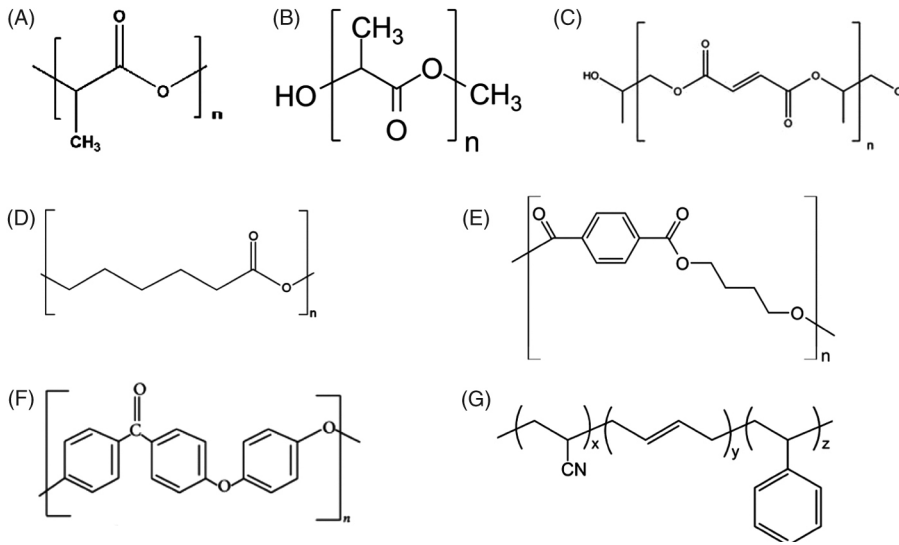

Figure 9.2 (A) PLA structure reproduced with permission from Ref. [54]. (B) PDLLA structure reproduced with permission from [55]. (C) PPF structure reproduced under creatives common at open access [56]. (D) PCL structure reproduced under creatives common at open access [57]. (E) PBT structure reproduced from open source Wikipedia. (F) ABS structure reproduced with permission from Ref. [58]. (G) PEEK structure reproduced under creatives common at open access [59].

will have a lower compressive strength compared to bone, which can be problematic because of its primary use in musculoskeletal tissue engineering. Despite these problems, PLA is still the best and most used materials in AM and tissue engineering for regenerative medicine application using FDM process.

9.2.1.2 Poly(D,L-lactide)

PDLLA oligomers functionalized with methacrylic chloride are being used with SLA technique for specific tissue engineering applications [67]. PDLLA has good biocompatibility and high mechanical strength: this has been shown by the successful implantation of PDLLA devices for bone fixation devices [68]. In this system, a composite sintered with poly-DL-lactide (PDLLA) and tricalcium phosphate (TCP) ceramic was produced. The TCP particles in a range of 30–60 wt% with 5 wt% increments were doped into the PDLLA matrix, which was prepared by melting and hot-pressing techniques for the reinforcement. Following scaffold formation, the structures were extracted with acetone and isopropanol to remove unreacted resin. This system aims at fixation bone after fracture. The fractured bone was gradually healed and the composite firmly and properly fixed on the fracture area during the implanted period, which provided a breeding environment for normal bone remodeling. In a similar system, PDLLA was functionalized with fumaric acid monoethyl ester and diluted in Nvinyl-2-pyrrolidone (NVP) to form a resin. Porous 3D scaffolds were printed through stereolithography of the resin and seeded with mouse preosteoblasts after processing. The seeded cells were attached to the polymer network and proliferation and cell death (apoptosis) throughout the scaffold were studied. [69] The more the hydrophilicity of PDLLA network, the better cell attachment and viability one could get. Tissue integrate on and cell engraftment could also be improved my creating a composite of PDLLA with natural polymers to match the mechanical properties of the site of implantation. The biocompatibility of PDLLA scaffolds along with the mechanical properties makes it a suitable candidate as a 3D printable scaffolding material.

9.2.1.3 Poly (propylene fumarate) (PPF)

PPF is one of the most extensively studied biodegradable and photo-crosslinkable polymers used in stereolithography [70]. Generally, PPF is mixed and combined with diethyl fumarate (DEF) as the solvent and bisacrylphosphrine oxide as the photoinitiator in SLA. The ratio of PPF and DEF is the most important factor in order to control the mechanical strength, the viscosity, and the printability of this biomaterial [71]. If the ratio of PPF/DEF falls below 50%, then the viscosity of the polymer and its mechanical strength will decrease too much to be printable. DEF solvent is the component that limits this process and ratio must be calculated and measured. Changes in polymer densities due to wrong ratios can completely destruct the final product. In a recent study [72], scaffolds using PPF were coated with β-TCP, synthetic bone mineral (SBM) or biphasic calcium phosphate (BCP), and bone morphogenetic protein (BMP-2) by using stereolithography process. These scaffolds were studies in vitro and in vivo in a rabbit model. This model showed bone regeneration inside the scaffolds with both ingrowth of native bone from the edges and generation of bone at locations on the interior of the scaffold for all three coatings. Inflammatory response was

low, which communicates a reasonable biocompatibility of these scaffolds and PPF. From this study, it is evident that PPF alone is not able to enhance cell activity and proliferation but also engraftment and osteointegration. The addition of BCP, β-TCP, and SBM loaded with BMP are necessary to enhance osteoconductivity and osteointegration [72].

9.2.1.4 Poly (caprolactone) (PCL)

PCL is a low-cost, biodegradable polyester that has been widely used due to the improvement of the FDM technique and the need for more biocompatible and biodegradable materials [73,74]. PCL rheological and viscoelastic properties upon heating are part of the best in terms of synthetic polymers and it makes PCL one of the best candidates for melt-based extrusion printing. However, its stiffness and extended degradation profile make PCL more useful in hard tissue engineering. PCL is stable in the body for 6 months and degrades fully in around three years, allowing it to provide support during healing and later be absorbed over time. PCL is therefore completely biodegradable (no toxicity in its degradation) and its biocompatibility has been proven in many studies [75]. PCL has been widely used in drug delivery devices so it has a shorter FDA regulatory path than other synthetic and natural polymer system, which is a huge asset for this polymer. For instance, a custom-designed airway splint device was printed using PCL, and administered to the patient under the emergency-use exemption from the Food and Drug Administration [76]. SLS uses also a lot of PCL beads in the size range of 10–100 μm. These beads can be melt-fused with laser heating [77]. To counter the drawbacks of stiffness and extended degradation, PCL is now being mixed with softer polymer [78]. An artificial blood vessel was 3D printed using a mix of PCL Chitosan and hydrogel. To overcome the problems related to previous use of autologous grafts using available synthetic grafts, the mix was created and studied in vitro and in vivo. To examine the optimum of the ideal vessel-like constructs, parameters were produced at 230°C. The maximum cell proliferation was obtained from PCL/7 wt.%CS/5 wt.%H and was tested by mitochondrial dehydrogenase activity. Overall PCL is a good candidate for tissue engineering when mixed as a composite with softer and more biodegradable synthetic or natural polymers.

9.2.1.5 Poly (butylene terephthalate)

Polybutylene terephthalate (PBT) is a thermoplastic polyester, like PLA and PCL. It is mainly used in FDM technique. The main drawback to PBT is not only its higher melting point (225°C) but also the fact that there are no obvious advantages over PLA or PCL. Therefore, PBT has seen much less studies in the 3D printing domain compared to the two other polyesters. Indeed, its biocompatibility and biodegradability are really similar to PLA and PCL. PBT is mostly used in the 3D printing field as a copolymer with PEOT. Poly (ethylene oxide terephthalate)/poly (butylene terephthalate) (PEOT/PBT) segmented block copolymers are widely used for the manufacturing of 3D-printed bio-scaffolds, due to a combination of several properties, such as cell viability, biocompatibility, and biodegradability. These block copolymers have a low viscosity at high temperature and are shear thinning, which is a high asset for the AM process [79]. PEOT/PBT has been used to increase the bone binding properties of the

implants. Filaments of PBT were also used in FDM to create bone scaffolds based on CT scans of canine trabecular bones [80]. PBT scaffolds can match the mechanical properties of the tissue that are implanted in and especially the porosity; this indicates a potential of creating biomimetic scaffold using PBT soon. These beneficial effects could either be a result of the coating and scaffold combination or the coating alone.

9.2.1.6 Acrylonitrile butadiene styrene (ABS)

ABS is a triblock copolymer. Its strength comes from the acrylonitrile and butadiene elements while its toughness comes from the styrene units. It is therefore less brittle than PCL or many polyester materials. Combining its good mechanical properties with its melting point of 105°C ABS is a good candidate for use in FDM and SLA techniques. However, surprisingly ABS does not perform better than PLA in most studies where cell integration and proliferation are needed. Therefore, ABS has been mostly used in cartilage engineering more than soft tissue engineering. Its cost is also relatively higher than PLA and PCL, which makes it less used in many studies [81,82]. However, the biggest problem of ABS is that it is not biodegradable, which is a major detriment in an industry that is moving toward resorbable materials.

9.2.1.7 Polyether ether ketone (PEEK)

PEEK is a semicrystalline polymer that has been used as biomaterial for AM to create rib prostheses, bone repair, and dental fixtures [83–85]. PEEK has many beneficial properties such as bio inertness, biocompatibility, and radiolucency that make it a prime material for bone replacement. Its low heat conductivity, and strength and elasticity come from the crystalline behavior of the polymer. Due to its high melting point, around 350°C, processing conditions for PEEK are more extreme than other polymers. Its crystalline behavior implies that crystals formation can be seen and therefore slow melting and cooling are necessary in order to control the structure created, this means that laser sintering is the only method that can be used with PEEK because of its high melting point, around 350°C, which has limited its application to only selective laser sintering [86,87]. However, this same property gives PEEK superior heat resistance, allowing them to undergo steam sterilization without softening. However, PEEK lacks the osteointegrative properties, biodegradability, biocompatibility, and cell attachment and proliferation that would make it a good candidate for soft tissue engineering and. Its biocompatibility is so low that it can trigger foreign body response and immune reaction when implanted: encapsulation, dislodging, and extrusion. Furthermore, PEEK implants are more expensive than many other polymer implants. With the greater speed, availability, and reduced cost of FDM other biomedical uses of PEEK are starting to be explored [88].

9.2.2 Hydrogel systems

Despite their tunable control, polymeric design lacks one major aspect, which is the biomimetism of natural ECM. Hydrogel-based 3D-printed construct would be able to mimic the complexity of natural ECM, improve cell growth by providing a 3D

scaffold, and provide cell support and delivery toward specific soft tissue. Hydrogels are crosslinked networks of hydrophilic polymers of various natural and synthetic polymers [89]. Hydrogels are three-dimensional polymer networks with the ability to hold a large quantity of water. They have been largely studied and used in the biomedical field into 2D and 3D structures for cell, biomolecule, or simply scaffold delivery. These hydrophilic polymer chains can be crosslinked chemically, physically, or ionically. Generally, the hydrophilicity and softness of hydrogels make them biocompatible and biodegradable materials because they have the potential to mimic tissue where they are implanted. To control this effect, the crosslinks play a major role. Hydrogels have been extensively modified to exhibit various chemical compositions, mechanical stiffnesses, levels of degradation, and structures. Recent studies [90,91] have shown that to reach the highest integration and viability of transplanted cells within a hydrogel, the biomaterial needs to match the materials and mechanical properties of the tissue.

Injectable hydrogels are a subgroup of all hydrogel but form a large part of those used in research and clinical biomedical applications [89,92,93]. Hydrogel inks formulated from injectable hydrogels must (1) flow under modest pressures (to protect the polymer structure), (2) gel quickly (to maintain the 3D structure wanted), and (3) maintain enough integrity after build up [94,95]. In situ cross-linking polymers may provide a middle ground between solid scaffolds and saline injections [96]. These materials are typically more gel or liquid outside the body and then become solid in vivo (temperature, time, light, UV, etc.), giving them injectable properties [97]. These polymers are typically thermosensitive or chemosensitive. Thermosensitive in situ cross-linking hydrogels experience a "reverse state change" where they are liquid at room temperature but solidify at body temperature [98]. Chemosensitive hydrogels crosslink due to a chemical reaction, resulting in a covalent bond as crosslink. The degree of crosslink, the gelation time, and the stiffness of the final product can all be controlled by changing polymer, crosslinker, or catalyzer concentrations.

Hydrogel inks are referred to as bioinks only when they contain cells and/or biochemical molecules. The 3D printing system, which is the most suitable for hydrogel printing is inkjet, light-assisted, and extrusion-based [99–101]. To design a hydrogel ink, a polymer solution that forms a network immediately after printing is needed. Networks can be physically or chemically (chemosensitive, thermosensitive) crosslinked. One main advantage to physically crosslinked hydrogels is their nontoxicity. Indeed, the absence of added chemical reduces the chance of an immune response from the body. However, chemically crosslinked hydrogels result in a covalent bond formation and therefore are more stable and controllable during the 3D printing process. Mechanical strength, shear strength, crosslinking density, elasticity, and volume changes can be controlled through the chemical crosslinking process more than physical crosslinked network. The number of hydrogels that can serve as bioink is still really limited but can be separated into hydrogels made from natural polymers (gelatin, hyaluronic acid, collagen, agar, alginate, fibrin) [102] and network made from synthetic polymers (polyacrylamide, polyurethane, PEG) [103] or a synthetic–natural mixture [104]. Gelatin, the main component of collagen, has been given much attention due to its natural origins in the ECM. It has the ability to suspend cells in a gel

at low temperatures [105–107]. However, glutaraldehyde is also being used often to create a stable structure and control the crosslinking. The main drawback of glutaraldehyde is the lower cell viability and proliferation in the scaffold despite the asset of controlling the process and structure [108,109]. Though stability of the hydrogel can be increased by longer glutaraldehyde crosslinking, it would also increase cell death on the periphery of the scaffold [110].

A triple network hydrogel composed by alginate and agar was created [111]. In this system, the toughness of the hydrogel is created by the entanglement of the alginate chains with the agar double network. This entanglement is restricting the agar helical chain. Crosslinking process and initiators are also being studied to compare the findings with previous chemicals. In that sense, Billiet et al. proposed the use of a biocompatible VA-086 as a photoinitiator compared to the conventional Irgacure 2959 [101]. High cell viability has been reported (>97%) for 3D printing of cell-laden gelatin methacrylamide (GelMa). Other novel processing strategies for commercial materials were found by Matrigel 115 and Pluronic F-127 [112]. Matrigel and mixed Pluronic and calcium phosphate cell-laden hydrogels were bio fabricated. A ceramic hydrogel was therefore created as a microtissue using a microfluidic device and a ceramic ink for robotic-assisted deposition. To bypass toxic effects of hydrogel crosslinkers, alginate has been used for controlled gelation across various printing techniques (extrusion, laser). Alginate has modifiable chemical and physical properties; therefore, attachment of cell-adhesive ligands can alter cell viability, proliferation, and differentiation meanwhile photo crosslinking ligands provide another means of gelation. Usually, alginate is gelated with calcium chloride ($CaCl_2$) solutions used as a chelating agent postprinting. These have shown impressing biocompatibility and processing characteristics as a cell delivery agent [113]. Fedorovich and others showed delivery of cells seeded in a scaffold by using high-viscosity alginate to deposit fibers of cell-laden hydrogel. They used a 3D plotting device into a petri dish containing $CaCl_2$ for immediate gelation [104]. Layer-by-layer inkjet-based printers have been adapted to print cell-containing alginate into a solution of $CaCl_2$. Viscosity enhancers, such as polyvinyl alcohol or hyaluronic acid, have been added to fix the position of the printed construct [112].

Supramolecular hydrogels are used in the AM of high-resolution, multimaterial structures. The noncovalent bonds allow the extrusion of the bioinks into support gels therefore to directly write structures continuously in 3D. Patterning of multiple inks, cells, and void spaces is possible with this system. In the biomedical field, the ability to create structures across length scales ranging from micrometers to millimeters and larger is fundamental due to the different scales in the body [49]. Another development has been recently shown of a hydrogel-based 3DP approach that allows the printing of shear-thinning hydrogel “inks” directly into self-healing “support” hydrogels. Both hydrogels are based on supramolecular assembly through guest–host complexes. This direct writing of guest–host hydrogels (GHost writing) can only be done because of the noncovalent and reversible bond's aspect of these hydrogels. Applying a physical stimulus such as stress can disrupt momentarily the hydrogel that will reform upon removing the stimulus [114,115]. They can therefore be used as injectable hydrogels [98], and as inks in extrusion-based 3D printing method. The receiving (guest)

hydrogel contains the same properties as a support matrix. It can deform to receive the extruded material from the guest hydrogel and then self-heal to maintain material properties. In this study, both hydrogels are based on modified hyaluronic acid (HA), which was selected for its amenability to chemical modification and for its biocompatibility [89].

Ghost writing needs shear-thinning and self-healing properties of both guest and host hydrogels. The dynamic bonds used in the support and ink gels bring these qualities, but may lack mechanical properties necessary for long-term stability or perfusion. To counteract this drawback, the gels can be designed to have a secondary, covalent crosslinking mechanism (e.g., light-mediated crosslinking). This secondary interaction (either the ink or support materials) will allow for stabilization of the material against perturbations [116]. Cellular constructs, which have dimensions that exceed more than a millimeter, may be good candidates for this approach. One can introduce channels to enable diffusion of nutrients, cell attachment, and removal of waste [117]. To accomplish this, methacrylate was introduced into the HA macromers, which were then modified with either Ad or CD moieties. These materials (Ad–MeHA and CD–MeHA, Fig. 9.3) have all necessary qualities for this approach: both supramolecular bonding (to enable GHost writing), covalent crosslinking (for mechanical stabilization), and induced by photopolymerization in the presence of UV light and a radical-generating photoinitiator. Importantly, the secondary modification does not affect the guest–host hydrogel properties prior to inducing covalent crosslinking. An increase of storage modulus (G′) measure with rheology can be seen following photopolymerization. Shear-thinning hydrogel inks were printed into self-healing support hydrogels, where the support gels now contained methacrylate groups (Fig. 9.3). As another illustration of the many functionality of this 3D printing approach inks with secondary covalent crosslinking were printed into support gels that did not undergo stabilization. A pyramidal hydrogel composed of six filaments with an average diameter of 260 was printed within a support gel. This new technique opens many opportunities in 3DP, including the printing of multiple materials and complex structures at high resolutions, as well as the formation of free-standing structures or constructs that have open channels, a similar technology can be seen in Fig. 9.3. In this study, hydrogels have been printed into GelMa crosslinked networks.

Another example of 3D printable hydrogel with extreme characteristics is a highly stretchable tough hydrogel, which is developed by combining poly(ethylene glycol) and sodium alginate, which synergize to form a hydrogel tougher than natural cartilage [119]. By adding biocompatible nanoclay, the tough hydrogel can therefore be 3D printed in various shapes without requiring support material. Cell viability is tested with encapsulating human cells, which maintain high viability over a 7-day culture period and are highly deformed together with the hydrogel. Biocompatible nanoclay is used in order to control the viscosity of the pre-gel solution by inserting it into the PEG – alginate hydrogel [120]. The nanoclay particles physically crosslink both with themselves and with the networks of the PEG and alginate to increase the viscosity of the pre-gel solution [121,122]. The biocompatible material's sodium alginate and poly (ethylene glycol) (PEG) can constitute an interpenetrating network (IPN). The IPN structure corresponds to the specific crosslinking process of both polymers.

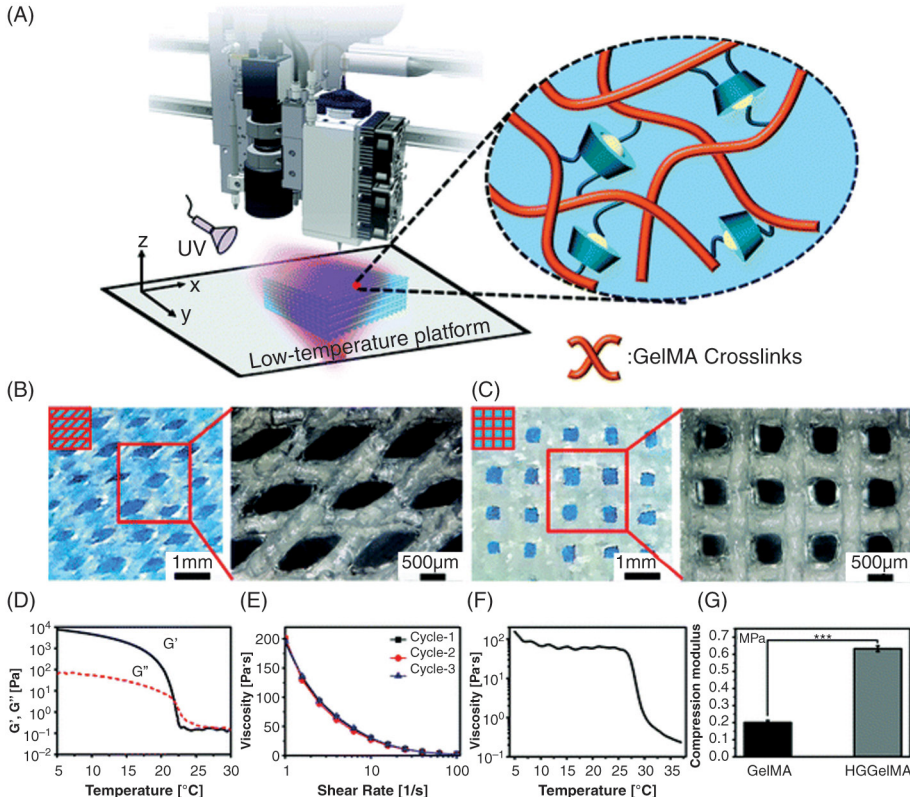

Figure 9.3 3D bioprinting of HGGelMAs into scaffolds using HGGelMA precursors as a printing ink. (A) Schematic of the 3D printing of HGGelMAs. (B and C) 3D rotational microscopy images showing the swelling equilibrium scaffolds under the swelling equilibrium (B, HGGelMA-45; C, HGGelMA-90). (D) Dependence of G′ and G″ on the temperature between the gel and sol states. (E) Shear thinning curves of HGGelMA ink via rotating measurements repeated for three cycles. (F) Curves of the viscosity of the ink as a function of temperature. (G) Histograms of the compression moduli of the GelMA and HGGelMAs. *Source*: Reproduced with permission from Ref. [118].

Indeed, an IPN is created when both polymers have selectivity in crosslinks and will only bind with themselves. The resultant hydrogel of covalently crosslinked PEG and ionically crosslinked alginate possesses high fracture toughness (due to the PEG characteristics) and allows cell encapsulation (due to the biocompatibility and attachment sites of alginate). The toughening of this hydrogel relies on a combination of two mechanisms: the reversible Ca^{2+} crosslinking of alginate dissipates mechanical energy, while the covalent crosslinking of PEG maintains elasticity under large deformations. The hydrogel can endure high stress in both tension and compression and has a fracture toughness above 1500 J/m^2, making it tougher than natural cartilage and yet with water content (≈77.5 wt%) that is tunable and within the physiologically

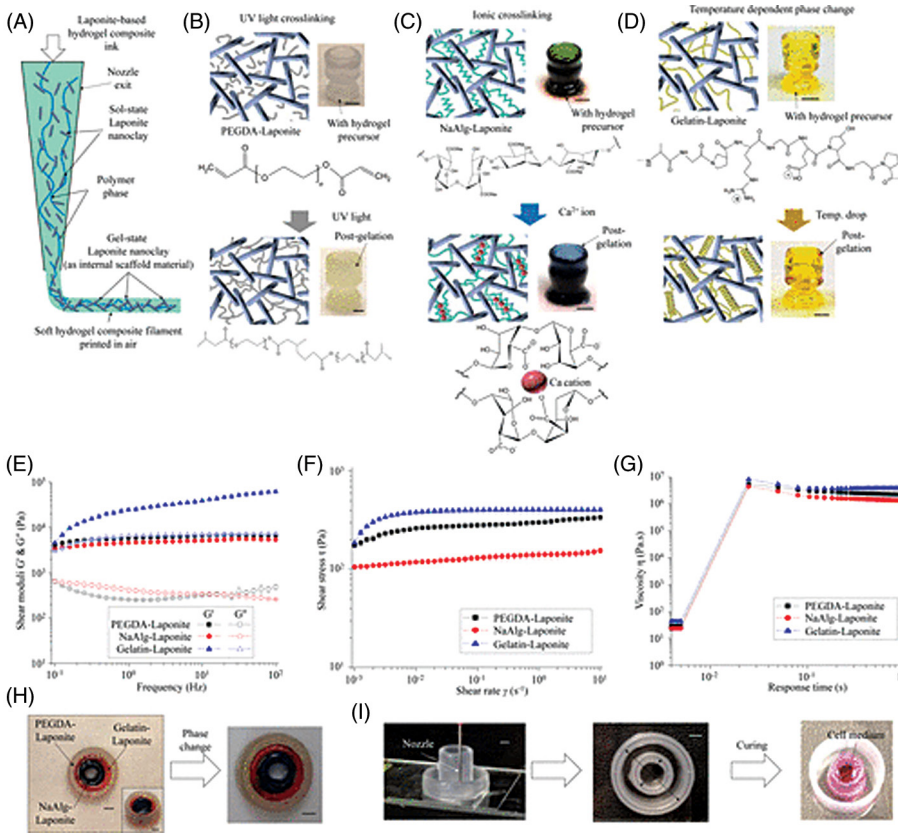

Figure 9.4 Soft hydrogel composite structure printing in air: (A) schematic of hydrogel composite structure fabrication where Laponite functions as an internal scaffold material. Schematics of interactions between pre- and postgelation hydrogel composite cups of (B) PEGDA and Laponite, (C) alginate and Laponite, and (D) gelatin and Laponite. Rheology measurements of three composite hydrogel precursor colloids: (E) shear moduli as a function of frequency, (F) shear stress as a function of shear rate, and (G) thixotropic response time. (H) Laponite-based triple-walled heterogeneous hydrogel composite structure. (I) Printed concentric cannular PEGDA_Laponite structure (all scale bars: 2.0 mm).
Source: Reproduced with permission from Ref. [123].

acceptable range. During printing, the pre-gel solution experienced shear thinning inside the extrusion needle, and quickly regained its viscosity upon exiting. Since the viscosity of hydrogel was enhanced by adding nanoclay, it was able to be printed into various shapes free from vertical limitation (example of such technology is given in Fig. 9.4). Controlling the concentration of nanoclay in the gel permits the viscosity to be optimized for extrusion-based printing while still maintaining 3D structures without requiring support material.

9.2.3 Inorganic and composite inks

Given the initial fabrication nature of 3D printing, the first applications of 3D bioprinting were to orthopedic designs and not soft tissue engineering. Powder-based selective laser sintering and laser engineered net shaping (LENS) was used for orthopedic implant research to avoid using metal implant, which is known to be nonbiocompatible and create immune response. [124] In contrast to soft tissue engineering, hard tissue scaffold designs typically use the powder-based SFF method to print ceramic, resorbable materials (e.g., hydroxyapatite) that contain varying degrees of porosity to promote cell infiltration and proliferation [125]. Due to their high stiffness, bioactivity, and biocompatibility (in certain cases) ceramic materials are widely used in biomedical applications. They can provide a natural and osteoinductive surface for cells and natural bone tissue development [126,127]. Currently, only direct printing of ceramics is available due to the difficulties of finding a liquid-phase ceramic and their really high temperature of melting (higher than the range of FDM printers) [128]. Ceramics are not responsive to light, and it is really difficult to create dense powder of them; therefore, SLA and SLS methods are not an option [129]. If thinking about only ceramics 3D printing method, only PB and inkjet printing are the two methods for direct printing of ceramics from powder and suspension form [130]. The only other option to use ceramics in 3D printing for tissue engineering is to combine them in a composite system making other methods such as FDM and SLA possible. At the beginning, 3D printing was focused on exclusively pure metals and polymers; however, as science pushed forward, the development of composite inks quickly emerged. Composite inks can enhance processability, printability, mechanics (stiffness), and bioactivity [131,132]. Below, we summarize the two most common ceramic-based inks and the three composite-based inks in 3D-printing: polymer-based, hydrogel-based, and ceramic-based composites.

9.2.3.1 Hydroxyapatite (HA)

The most important part of ceramic 3D printing in the biomedical field is to mimic all characteristics and properties found in natural bone (minerals, structure, mechanical properties, etc.). Therefore, HA in powder form is widely used in AM because of its abundance as a calcium phosphate phase in mineralized bone [133]. 3D printing HA is performed by layer-by-layer technique where poly (acrylic acid) solutions are sprayed onto HA powders followed by sintering to compete the solidification process. At the end of the process, HA powder binding can have strength really close from the bone (0.5–12 MPa). In vivo studies have been performed into mouse models. It shows growth of new bone on the edge of the implanted scaffold but also osteoid formation and cell migration inside the scaffold. Scaffold enhances also vascularization and cell attachment due to its high biocompatibility [134]. However, the autograft control treatments performed still better than the scaffold implanted (showing regeneration after less than 8 weeks compared to more than 10 for the scaffold). To reach clinical treatment, more studies must be performed. New HA-based scaffolds with 70% interconnected porosity have been created. These new scaffolds have promising results not only in terms of osteogenic stimulation of progenitor cells but also in terms

of potential treatment and clinical trial applications [135]. Other methods of highly concentrated HA inks have been created. These scaffolds can be self-supporting with minimal organic content. Most of the research and studies performed on 3D printed HA is useful only for the bone regeneration field [136].

9.2.3.2 Tricalcium phosphates (TCP)

Variation of α- and β-TCP is another type of calcium phosphate powders that is used in 3D printing [137]. Tricalcium phosphates are the second most abundant calcium phosphate phase found in the human skeleton. Usually, β-TCP is used more than other has a calcium phosphate phases because it has a faster resorption rate in the body. Phosphoric acid is the most common binder used with TCP. It partially dissolves the calcium salt, allowing it to recrystallize and form new bridges between particles upon drying [138]. As for HA, particle size, binder droplet size, and scaffold geometry must be clearly controlled in order to be able to create TCP scaffold with specific resolution, porosity, and strength. TCP alone shows great properties in vitro but in vivo studies have shown that, as HA, autograft still perform better. The answer to this problem is to create β-TCP with polymer additives that aim to improve the binding properties of the final scaffold [139].Adding a polymer component to TCP (being synthetic or natural) will enhance the cell viability and attachment, which can be low in TCP. Furthermore, polymer scaffold shows better osteogenic and mechanical properties in some instance and can help cell migration into diseased tissue. For example, β-TCP has been combined with PCL only to improve interlayer binding and scaffold mechanical properties. Addition of pharmaceutical agents, like alendronate, on the surface of TCP has been used simultaneously to improve the wound healing response in vivo and enhance healthy cell proliferation [140]. Adding a naturel polymer component to TCP could be the perfect candidate to induce higher regeneration. Mixtures of TCP and alginate powders have been created for this reason. The addition of alginate (with phosphoric acid as the binding solvent) can improve the mechanical properties of the scaffold by improving its stiffness and reducing its brittleness. TCP-alginate promotes osteoblastic cell proliferation, migration, and viability compared to only TCP scaffold. The last important factor of adding a natural polymer, in this case alginate, is the lowering of the probable immune response and side effect created during the implantation of the scaffold. One last example of natural polymer added to a β-TCP scaffolds is collagen via ceramic SL and gel casting. The aim of the project was to develop osteochondral scaffolds for tissue engineering [141]. Overall, TCP scaffolds perform similarly to HA. They are both good base material for 3D printing in the bone regeneration field.

9.2.3.3 Ceramic-based composites

TCP and HA can also be used together to create a composite ceramic-based scaffold. The two powders can be formed together in the printing bed. These ceramic composites show the same cell viability, proliferation, and overall immune response as HA and TCP alone. By changing the mixture concentration and adding more TCP but also coating the scaffold with PLGA or other polymer (PCL) mechanical properties of these composite can be really close to natural bone [142]. Another important factor to take

in consideration is the need of organic solvent binders for these composites. This can be a big drawback for certain stem cells that cannot handle organic solvents. Other studies show that composite of ceramic can have limitation in terms of bone formation and osteoconduction compared to calcium phosphate alone. New studies should be performed to enhance the ability of both components of the composites to create a new 3D printable bioink, which could have better properties than natural bone for regeneration. Adding a polymer into this composite still seems to be the ideal answer to enhance regenerative properties. Another study shows the addition of poly (vinyl alcohol) (PVA) to HA. In this case, the final properties of the product were way lower than cancellous bone but the process and techniques of 3D printing were a lot easier. Indeed, adding PVA reduces the sintering temperature and creates better binding between layers. Adding this polymer offers a more robust and easier to handle final product. Using SLS or PB as a fabrication process to create a good artificial scaffold, which is made only of ceramic composite, should be studied. However, the addition of a polymer part in the composite shows already higher and better properties in terms of regeneration. There is evidence that the pore size and geometry play a role in scaffold performance and can enhance the ability of HA ceramic scaffolds to accelerate healing [133].

9.2.3.4 Polymer-based composites

PLA is one of the most used bioinks in 3D printing. However, PLA promotes cell adhesion but does not support cellular activity. To counteract this drawback, it is widely performed to mix a bioactive ceramic to PLA in order to promote cell proliferation [143]. One example of a polymer-based composite is the well-known PLA-PEG or PLGA-PEG with a ceramic. Adding a PEG as a plasticizer will improve greatly the biocompatibility and hydrophilicity of the composite while the calcium phosphate glass will act as a bioactive carrier to improve cell migration and proliferation. This printable polymer-based composite bioink is used in DIW to make bone scaffold. The regeneration process is done by the culture of human mesenchymal stem cells (MSCs) [144,145]. MSC cultured on these scaffolds shows higher integration, engraftment, viability, differentiation, and proliferation than on control surfaces (such as Matrigel or pure PLA-PEG). This suggests the potential for a better regeneration in vivo due to the addition of a calcium phosphate to the composite by inducing more cell migration and healing in vivo. The addition of a glass changes the chemical and topographical structures of the scaffold surface in ways that seem to benefit cell progression and proliferation. Another aspect of polymer-based composite is the specific surface erosion of PCL within the body. It makes it an interesting system for drug and growth factor delivery in 3D printed scaffolds. Indeed, the slow degradation of PCL allows for the control of the release (compared to bulk degradation being difficult to handle) [146]. PCL fibers with poloxamine and dexamethasone (DXMS) have been printed using FDM. The study shows the release prolife of both materials and the effect they have on MSC development and viability. The addition of DXMS as a composite on the PCL shows greater cell proliferation and differentiation than PCL alone. PCL has also been mixed with HA particles under an extrusion process to form filaments for FDM. The goal of this study was to print inside goat femoral condyle and promote regeneration

after trauma [147]. The scaffolds were also coated with PLA/PLGA to mimic the osteochondral interface. After 10 weeks, smooth, homogeneous, and integrated articular cartilage was observed. PCL has also been dissolved in DCM and THF and used with HA powders and carbon nanotubes to enhance the osteogenic behavior of cells in the scaffold. Direct ink writing with PCL in these composites has been performed. All studies show that adding a ceramic of component will improve the cell proliferation and differentiation while the PCL itself will sustain the cell viability. The pores that were reached in this study were about 200–700 μm, which corresponds to pores needed for cell migrations in the bone to induce regeneration [148]. The addition of carbon nanotubes increased the mechanical properties and added conductivity to the scaffolds; MG63 cells attached to the scaffold surface due to the presence of PCL and proliferated due to the presence of the carbon nanotubes.

A characterization of bone tissue scaffolds fabricated via 3D printing from hydroxyapatite (HA) and poly(vinyl)alcohol (PVOH) composite powders has been performed [41]. Flowability of HA:PVOH precursor materials affected mechanical stability, microstructure, and porosity of 3D printed scaffolds. Compressive strength testing showed anisotropic behavior of constructs and part failure at the boundaries of interlayer bonds. There is a trade-off between the ability to remove PVOH with thermal degradation products during sintering and the compressive strength of the final product. The ultimate compressive strength of 55% porous green scaffolds printed along the Y-axis and dried in a vacuum oven for 6 h was 0.88 ± 0.02 MPa. However, the pores of 3D printed constructs could be user designed because bulk interconnectivity was not ensured in the study. The imperfect packing of powder particles created a surface roughness and nondesigned porosity within the scaffold. All these features are considered promising and can promote cell proliferation and adhesion in vivo due to the composite behavior of the scaffold. Fig. 9.5 shows the SEM and final structure of the composite created via this method.

9.2.3.5 Hydrogel-based composites

One problem with pluronic hydrogels is that they have a low strength and inertness with respect to osteoblasts. Therefore, they are usually not used alone in DIW but more in a hydrogel-based composite. The mix of pluronic hydrogel with a bioactive glass provides an increase in strength as well as a bioactivity that was not present in the hydrogel alone [149]. Another type of composite is to use hydrogel-based 3D printing process and add a co-printing process with a second, stiffer polymer. This can allow for stiffer hydrogel to be created and the second polymer can have different properties in terms of cell interaction. One system that was performed this way is hyaluronic acid combined with UV-curable glycidyl methacrylate. Tissue growth, cell viability, biocompatibility, and low immune response were shown during the implantation of this composite scaffold into a porcine mandibular model [150,151]. Most of the scaffolds show good cell attachment and proliferation when cultured with cells. One important aspect of the hydrogel-composite ink is the improvement of mechanical strength since hydrogel is mostly composed of water and usually soft. In this study, increased mechanical strength was achieved through the addition of PVA

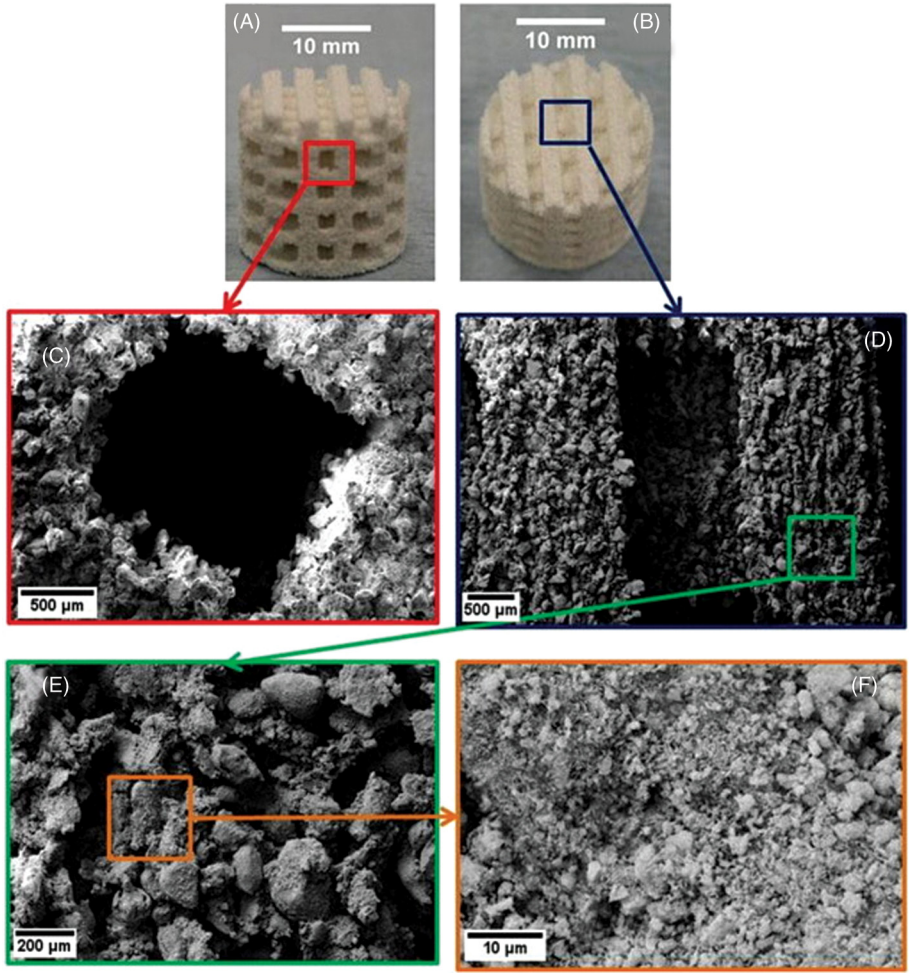

Figure 9.5 SEM micrographs were taken to show the typical morphology of designed struts, pore channels, and the surface topography. Structure of 50 wt.% HA scaffold printed along the *X*-axis. (A) and (B) Printed structure, (C) designed pore channel, (D) scaffold strut, E and (F) scaffold surface.
Source: Reproduced with permission from Ref. [41].

to the individual Sr-MBG particles together, decreasing the scaffold brittleness and increasing stability in solution. Finally, bioinks of hydrogel combining metal powders have been developed for DIW printing of mechanically stiff, porous scaffold systems [152]. These powder-based liquid inks are a variety of metal powders mixed with polylactic-co-glycolic acid (PLGA) in DCM. PLGA being extremely biocompatible and biodegradable, the cell viability, activity, and proliferation were already high before adding the metal powder. The goal of these powders is to enhance the mechanical properties of the hydrogel. These inks could be printed through a 200-µm nozzle to

form porous, strut-based biomedical scaffolds. The rest of the process is more difficult and with higher pressure than other composite because of the metal powder part of the composite. The products are subjected to H2 thermochemical reduction to sinter the 3D object to its final state. In general, hydrogel-based composites used the benefit of biocompatibility and biodegradability of the hydrogel while improving its mechanical properties with stiffer materials.

9.3 Modeling and architecture design of scaffolds

Most works performed with 3D printing for tissue engineering include simple structures such as mesh, squares, and geometric shapes. These structures have sharp edges and corners–these shapes are usually obtained through Boolean operations. The sharp parts of these scaffolds often imply edge effect and different cell or molecule interactions that could be negative for the regeneration process. A new way of thinking about AM of scaffold for tissue engineering would be to create structures without edges. One example that has recently been used is hyperbolic surface or triply periodic minimal surface (TPMS). One major aspect to these new types of structures is their high surface to area ratio. The larger the surface and the better cell attachment, cell proliferation, cell viability, and cell activity, all which are the basic principle for regenerative medicine. This high ratio also improves the porosity of the scaffold and its mechanical properties [153–155].

Kapfer et al. [156] were able to observe two different types of scaffold design that can be generated with TPMS unit cell libraries as seen in Fig. 9.6C. In this work, they created a sheet solid architecture and a network solid architecture. These two types of solids are based on minimal surface sheet/network solids. They were able to show that sheet solids have a higher elastic and bulk modulus than network solids, for the same solid volume fraction. The sheet solids also offer a larger surface area for cells to attach. Using modeling, they were able to show some new scaffolds that could be built to improve regenerative process. Other method of 3D modeling before AM has been performed as seen in Fig. 9.6A, B. These virtual prototypes of internal and external structures allow for an insight of the perfect structure and scaffold as it should be before 3DP. Stress–strain curves can be measured before starting the process, and different scaffold architecture can be virtually tested before starting the manufacturing process.

The K3DSurf software was used to generate scaffolds based on gyroid (G) and diamond (D) architectures [158,159]. Pore size and porosity were then calculated via modifying equations in the z-axis. Gyroid structures present strain and stress curves that are much more homogeneous over the entire structure compared to normal cubic scaffold. Cells being responsive to the deformation of the matrix, gyroid structures will offer more adherence and migration to cells compared to a cubic lattice. These structures present optimum mechanobiological stimulation. A computer-designed gyroid architecture made by stereolithography has been performed in this study. It was compared with a random pore architecture resulting from salt leaching. They found out that the scaffold structures showed similar porosity and pore size values, but the gyroid type showed a 10-fold higher permeability, due to the absence of size limiting

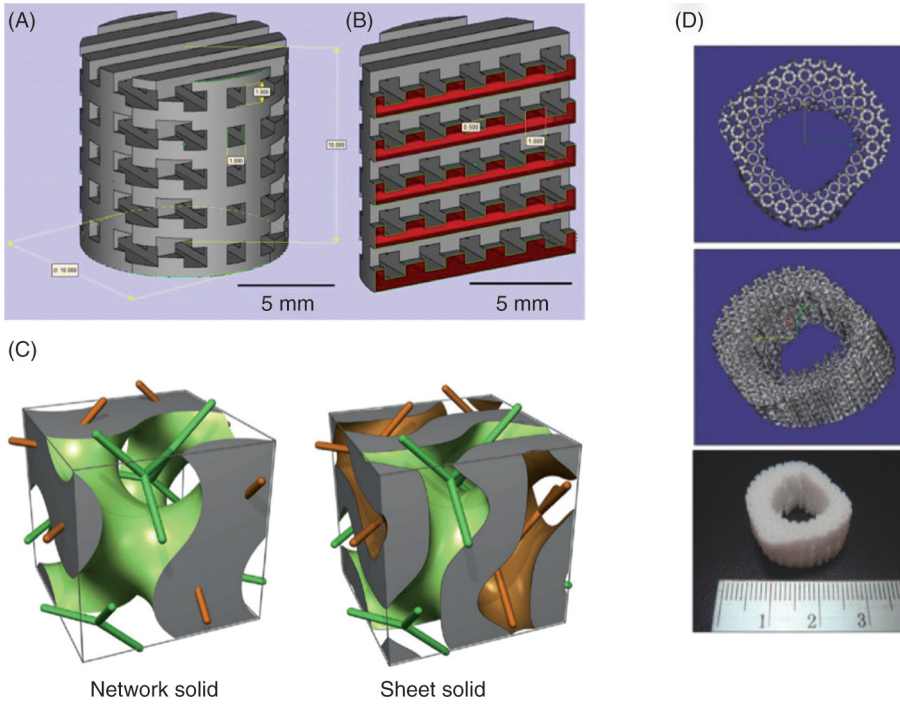

Figure 9.6 (A and B) CAD design of 3DP cylindrical scaffolds (external and internal). Reproduced with permission from Ref. [41] (c) Scaffold designs of 50 % volume fraction derived from the gyroid minimal surface. Left: Network solid architecture. Right: Sheet solid architecture. Reproduced with permission from Ref. [156] (D) Virtual and physical prototypes of the functionally graded porous scaffold of a femur bone segment. Modified and reproduced with under creatives common attribution law [157].

pore interconnections. This result has a great impact on cell seeding, culturing, and proliferation due to difference in permeability. One could therefore control cell seeding upon different gyroid scaffold to control its characteristics and adherence.

The first finding about computer-controlled fabrication, modulation, and mechanical characterization was performed on simple cube models [160]. These cubes were based on TPMS and manufactured with a layer-based fabrication device. The results show that computer-controlled fabrication can lead to not only control over blood and nutrient supply but also proliferation, viability, and adherence of cells. These seeding–feeding networks show the reason behind the natural choice of TPMS forms in biological systems. By biomimetism, computer-based fabrication can replicate the specific architecture of already existing biological system to apply them to tissue engineering and regenerative medicine. Other solution based on TPMS to create computer-based porous scaffold has been studied [161]. This work is based on the periodic surface model applied on the implicit surfaces of TPMS. TPMS generation was performed by describing them with periodic surfaces with simple trigonometric functions. Various chemical, mechanical, and

physical applications result from this study. Pore size, pore distribution, permeability, cell adhesion, and proliferation can therefore be controlled in a novel way due to these implicit surfaces of TPMS. All the hexahedral meshes already presented in the conformal refinement of TMPS suggest that this modeling approach has practical applicability, corresponding to existing patterns in the human body. This novel modeling method was successfully validated through many designs of bone scaffold models.

A new method for the design of 3D porous scaffolds, based on a hybrid method of distance field and TPMS, has been studied [162]. An almost defect-free scaffold was created by using a traditional distance field algorithm and adding Boolean operations of TPMS-based unit cell. The high quality of the external surface and the specific anatomy model were obtained without time-consuming and trimming or remeshing process. A heterogeneous porous scaffold can be obtained in a similar way. The porosity, internal architectures, and the range of heterogeneity can be precisely controlled using TPMS in a computer-based modeling. In addition, they could create and determine the internal architecture type and porosity at the spatial locations, uniquely and continuously within complex 3D anatomical shapes. Another advantage of the method is the possibility to control the pore size distribution without changing the size of hexahedral structure. It allows also to control pore size while maintaining perfectly interconnected pore networks. Another example of controlled and computer-based scaffold modeling for tissue engineering was performed by Yoo [163]. He presented a general design framework for 3D internal scaffold architectures to match mechanical properties and porosity simultaneously, by adding an implicit interpolation algorithm based on the radial basis function (RBF) [164]. This work has high impact on the modeling of 3D scaffold that can be processed through AM and has applications in the biomedical field and tissue engineering. While using Boolean operations based on the distance field and TPMS-based unit cell libraries, Yoo focused on using the RBF to create an automated porosity distribution control. He was able to produce highly porous and heterogeneous structures matching the required anisotropic stiffness.

Other works on computer-based 3D modeling include not only the creation of software and libraries but also tools for other to use. An open source software tool for the design of scaffolds [165]. Different highly complex geometric models with different levels of porosity and permeability can be obtained. The tool and software are still based on TMPS and the results of this study show that it is possible to create structures with higher levels of porosity than ever created before.

One of the most important aspects in tissue engineering is to match the mechanical strength, stiffness between the 3DP scaffold, and the targeted tissue in vivo. The porous structure of bones and their stiffness can be separated into two gradients: radial gradients in long bones, and linear gradients in short and irregular bones. Creating a functionally graded scaffold is therefore an important step to use computer-based modeling of 3D scaffold for regenerative medicine. A study shows that they were able to create a radial gradient design by arranging cylindrical unit cells in a concentric manner. In that case, the porosity decreases linearly from the center to the periphery [157]. Therefore, by adjusting the porosity and stiffness, scaffold with different linear and radial gradient can be created in order to match the properties of bones in vivo [166]. The same group was able to use this method in order to fabricate a human

mandibular cancellous bone scaffold and a femur bone segment, both with functional gradients using SLS [167]. Fig. 9.6D shows an example of a functionally graded femur bone segment. This process is highly accurate and reproducible. Another way of designing a gradient structure is to use a shape function and an all-hexahedral mesh refinement [168]. A truncated bone is subdivided and represented using various irregular hexahedral elements. Each of these elements is converted into a pore element based on the shape function. After doing the union of all operations among the irregular pores, the entire model is created. The bone scaffold is finally obtained by calculating the difference between the contour model and the pore model. A computer-based pore size distribution can therefore be achieved for bon scaffold in tissue engineering.

Computer-based modeling can also focus on the different processes available in 3DP. We cover here modeling for FDM and SLS. To be able to apply computer-based modeling to these processes, the parameters and limitation of AM must be studied. The main parameters for SLS are part-bed temperature, laser power, and scan speed. An example of why modeling could allow for more control and understanding of SLS is in the case of the polyetheretherketone/hydroxyapatite (PEEK/HA) system. Studies show that in this system, HA should be kept at 40 wt% or below else the structural integrity will vanish. To keep well-defined pore interconnectivity and good structural integrity in the polyvinyl alcohol/hydroxyapatite (PVA/HA) and polycaprolactone/hydroxyapatite (PCL/HA) systems, HA should be kept at 30 wt.% or below [169]. Another limitation in the SLS process is material wastage when building small prototypes such as tissue-engineering scaffolds. One way to overcome this problem is to add a compact adaptation system into the SLS part bed allowing for more transfer of the motion of the SLS into its own small part bed. SLS has other limitations, for example the low retention of cells during the cell seeding, which is a major issue in tissue engineering. Th main reason is that the material used in SLS is mainly synthetic and not natural (which would promote cell attachment). The pores created in SLS are also much larger than cells and therefore cells just fall through the pores without being retained in the scaffold nor attach during the seeding process. Different methods of process have been studied to counter these issues, for example, using composite 3D scaffold with adding natural polymer to promote cell attachment and reduce the size of the pores. An alternative solution is to inject cell-laden collagen hydrogel into the porous structure.

All those limitations show that more studies should be performed on the process themselves before even thinking about the bioinks and the different new materials available. A study has been made on modeling the process of SLS and understanding where the limitations are and where to fight them. Fig. 9.7 shows the science behind SLS by modeling and performing finite element analysis on the entire system. PCL being mostly used for FDM and SLS the modeling shows that the pressure drop and the velocity of the PCL melt flow depend on the flow channel parameters [170]. The temperature gradient of PCL shows that it liquefies within 35% of the channel length, which means that it is already melted for the rest of the channel. The SLS process and especially the heat transfer in SLS have been modeled. This model includes thermal conductivity, thermal diffusivity, surface reflectivity, and absorption coefficient. This modeling helps understand which bioink and laser beam properties are showing the best sintering results [171].

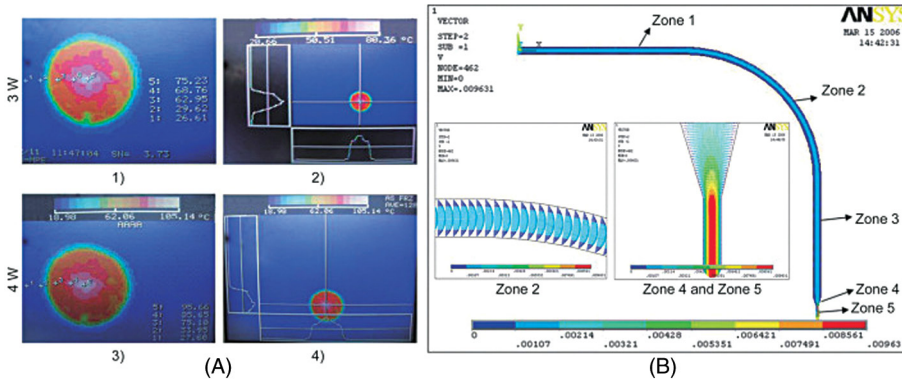

Figure 9.7 Process modelling. (A) Temperature distribution and Gaussian contour in the laser sintering process: (1) and (3) are temperature distributions; (2) and (4) are Gaussian contours. (B) Velocity profiles at different zones along the melt flow channel.
Source: Reproduced with under creatives common attribution law [157].

9.4 Future and concluding remarks

Biomanufacturing with additive manufacturing process is already an intrinsic part of tissue engineering field and regenerative medicine. Due to its many advantages like creating composite-based scaffold, matching the mechanical properties of the in vivo tissue or being able to precisely create architectures with pore distribution favorable for cell seeding it plays a major role in the start of regenerative medicine. A multitude of bioinks can be chosen: polymers, hydrogels, ceramics, and composites to allow for specific needs in different tissues in the body. Cell encapsulation, viability, activity, proliferation, and attachment can be precisely controlled by the architecture of the scaffold created during the AM process. Tissue engineering using 3DP will become a solution for many diseases due to its ability to inject nontoxic material including healthy and regenerative cells. Moreover, the architecture of scaffolds can be modified as wished to match the structure of the in vivo tissue. As 3D printing and polymer science matures, using them for tissue engineering is positioned to be the key approach of healing many diseased and damaged tissues.

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3D bioprinting of tissue systems

10

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Chapter outline

10.1 Introduction

The saying "Alone we can do so little, together we can do so much" by Helen Keller is meticulously followed by our human body as the functionality of it depends on the interplay of different types of cells, which results in the hierarchical level of organization in the body like tissue and organs. Tissue is a group of similar kind of cells with their extracellular matrix (ECM) and is specialized to perform a single function [1–3]. Accidental injury or trauma/disease can result in the loss or damage of a part of the tissue consequently leading to loss of its function. In mid-1980s, tissue engineering laid its foundation and in the last decade has spread its roots to emerge as a

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potential prospect for repairing or restoring the tissue loss [4–8]. Various techniques like freeze drying [9,10], particulate leaching and solvent extraction [11–14] electrospinning [12,15], gas foaming [16,17], cryogelation [18,19], etc. have applied the principle of biology and engineering to synthesize biodegradable and biocompatible three-dimensional (3D) scaffolds to restore the native structural and functional properties. However, none have been able to meet the requirements and the void still needs to be fulfilled. The designed scaffolds lacked the intricate architecture of the 3D tissues and the manual methods adopted for cell seeding, after the synthesis of scaffold, could not achieve uniform distribution of cells [20–23]. In the last few years, major thrust has been placed in the field of 3D printing because of its automation, speed, accuracy, reproducibility, and simultaneous seeding of scaffolds with uniform distribution. The use of high-throughput technique and computer-aided designing enabled it to synthesize precise 3D structures [24–27].

3D printing also known interchangeably as solid-freeform fabrication (SFF), rapid prototyping (RP), additive manufacturing (AM) ensures desired alignment and printing of either single cell or cell aggregates with media and appropriate carrier (bioink) on the substrate (biopaper) [28–30]. The bioink (approximately 10,000–30,000 cells per 10–20 μL of droplet) prepared should be biocompatible and facilitate cell proliferation and adhesion resulting in mini tissue formation [31,32]. The technique involves layer by layer addition of a single unit (cells and scaffold) to eventually result in the formation of the anticipated 3D tissue like structures. The complex 3D structure of a tissue including tortuous path, cantilevers, voids, etc. are represented by usual 2D structures stacked upon each other like circles, points, and lines [33]. The widely used bioprinting methods are based on the principle of jetting, extrusion, or laser-induced forward transfer (LIFT). The major limitation in achieving successful 3D implant is the vascularization of the construct as it has already been well established that diffusion of oxygen and nutrients is limited to 1–2 mm [34].

10.2 Scaffold-based approach

The cells need a supporting and stimulating microenvironment for them to adhere, proliferate, and differentiate. Therefore, the materials preferred for the synthesis of scaffolds should have properties like the ECM of the particular cells seeded. The ECM allows the cells to adhere and proliferate enabling them to form cell–cell contact leading to their differentiation [35–37]. In return, the cells and certain enzymes initiate ECM remodeling, a prerequisite for development, wound healing, homeostasis, etc. [38,39]. Apart from this, the synthesized scaffold should also accomplish other requirements like optimized pore size and pore connectivity for proper cell adherence and proper diffusion of nutrients and gases. It should be biodegradable and biocompatible so that the degraded products are nontoxic and do not interfere in the long-term implantation. Moreover, the degradation of the biomaterial should be fine-tuned such that the synthesis of the neotissue is coordinated with the degradation of the scaffold. Too early degradation of the scaffold would not provide the initial support required by

the cells for their functional activity whereas too delayed degradation of the scaffold would cause stress and tension on the synthesized tissue. The scaffold should also possess desired mechanical strength so that it could withstand the forces applied by cells [40,41].

10.2.1 Polymers

Polymers are one of the most prospective candidates for synthesis of scaffolds as the hydrogels/cryogels synthesized by them have high water retention capacity, which mimics the native microenvironment of the tissues. The use of both natural and synthetic polymers is widely accepted depending on the desired application. Natural polymers have characteristic similar to the natural ECM and thus closely resembles the microenvironment facilitating enhanced cell proliferation and differentiation [42–46]. Natural polymers like gelatin, collagen, laminin, chitosan, etc. have shown potential in the field of bone, cartilage, skin, neural, and cardiac tissue engineering [47]. Agarose–Matrigel hybrids have been synthesized using dual syringe 3D printer for encapsulating human intestinal epithelial cells HCT116 and it resulted in proper adhesion, growth, and cell–matrix interaction [48]. Using 3D printing helps overcome bottlenecks of conventional cell culture technique as explained in Fig. 10.1 [49].

However, the synthetic polymers like poly(ε-caprolactone) (PCL), poly(D,L-lactic-co-glycolic acid) (PLGA), poly(ethylene glycol) diacrylate (PEGDA) can be manipulated for better process ability, controlled degradation, and mechanical properties [50]. It has been shown that 3D printed polycaprolactone (PCL) trachea scaffold cultured in chondrocytes suspension for either 2 or 4 weeks showed similar native architecture reconstruction compared to its control. Moreover, when transplanted subcutaneously in nude mice attained properties similar to mature cartilage tissue [50].

10.2.2 Metals

Metals, known for their high mechanical strength, have been widely used in the field of bone tissue engineering [51]. Metals not only provide mechanical support but have also shown to promote bone tissue formation and growth. The most accepted metals used for medical and dental implants are titanium and cobalt-based alloys, stainless steel [52–54]. However, these implants suffer from limitations like lack of cell adherence properties, long degradation times, release of toxic ions on corrosion and thus inducing inflammatory response. In order to mimic the properties of bone, use of porous metals like Mg-based, Fe-based, Ca-based, Ti-based, Zn-based, and tantalum have gained huge attention over the years. Thus, introduction of “biodegradable metals” have proven to be an alternative to these limitations [53,55,56].

10.2.3 Ceramics

Ceramics have the inherent property of both metal and nonmetal and its role has been well established in the field of both soft and hard tissue engineering. However, the property pertaining to its high mechanical strength makes it highly suitable for bone

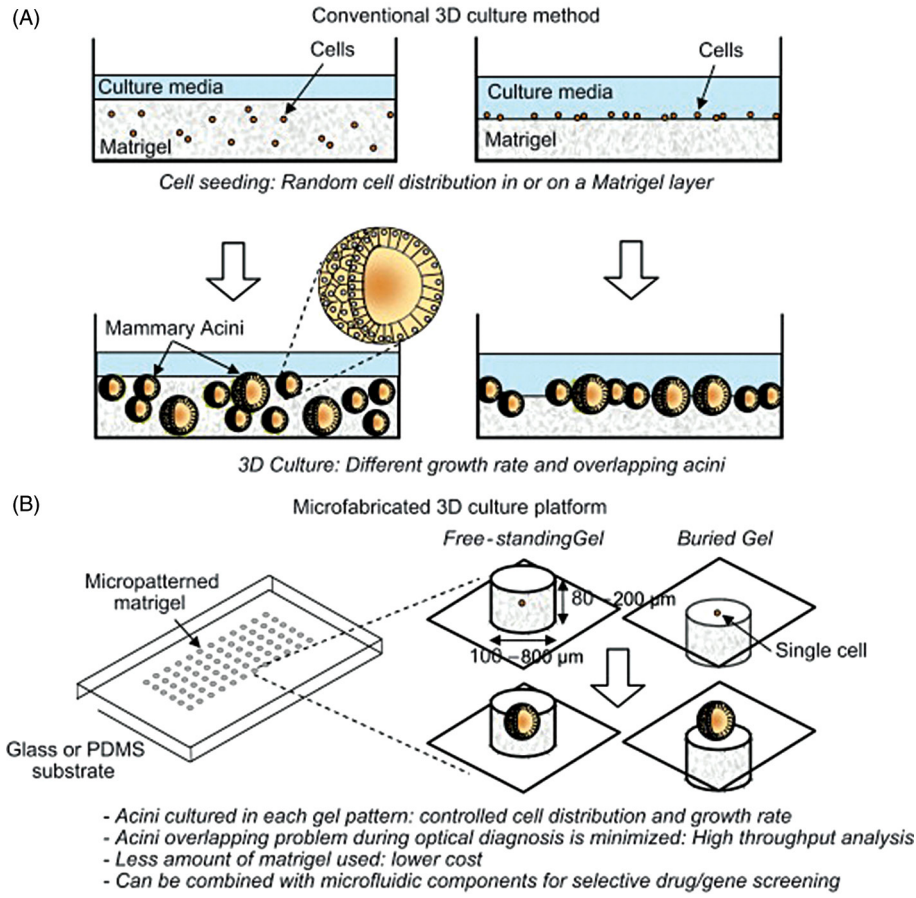

Figure 10.1 Schematics comparing traditional 3D culture setup to microfabricated 3D culture platform. Normally in 3D cultures, cells are haphazardly distributed within the Matrigel, resulting in overlapping that cause cell growth in different rates, thereby making individual analysis difficult. Additionally, Matrigel and other reagents used are very expensive that stops large-scale cellular studies. Whereas, in a microfabricated setting, one can achieve an organized array of cells patterned on Matrigel that can be used for screening of various drugs and biochemical arrays.
Source: Published with permission from [49]).

tissue engineering. The various examples include hydroxyapatite, calcium silicate, tricalcium phosphate, which have shown to provide increased mechanical strength and porosity to the incorporated scaffolds [57–59]. Bioceramics can be classified into three types: (1) inert, (2) bioactive, and (3) resorbable. Among these, the bioactive and resorbable types are widely used for tissue engineering applications as they degrade over the period and also promote cell adherence and proliferation due to their inherent structural and chemical properties [60]. αTCP/collagen-based cell laden scaffolds

were synthesized using 3D printing and have shown increased mechanical strength and mineralization compared to their cell-laden collagen scaffolds and alpha TCP/collagen scaffolds dipped in cell solution [61].

10.2.4 Composites

Composites, as the name implies, is the combination of two or more of the above-mentioned materials for the construction of the desired scaffold. The composites can be prepared either through proper blending of the materials to produce a homogenous scaffold or by the incorporation of one of the material types as nanoparticles/microparticles to increase the efficacy of the construct. Thus, use of these composites can pave the way for improved tissue engineering [24,62–64].

10.3 3D printing techniques for scaffold fabrication of tissue construct

3D printing has been extensively studied and used in the manufacturing industry and now its role has also expanded to the field of medical technology for the synthesis of customized implants, study of various drug molecules, presurgery operations, which can also be performed on the in vitro synthesized scaffolds which resembles closely in microscale and nanoscale to the in vivo tissue architecture [65,66]. The layer-by-layer synthesis of 3D tissue like construct is completed using high tech computer-aided designing wherein the cross-sectional images of MRI or CT scan of the anticipated tissue/damaged part of the tissue is compiled to form a 3D image. The 2D slices assimilated from the complex tissue architecture by the imaging techniques are fed to the software, which converts the information to a standard template library (.stl) format, which can be read by the printer and thus using the "bottom up" approach results in the synthesis of the entire 3D scaffold mimicking the 3D tissue. The .stl file also gathers information regarding color, thickness, texture of the 3D printed construct. Precise positioning of the cells in the in the *X*–*Y*–*Z* axis by the printer head helps in detailed and accurate cell seeding for 3D scaffold formation [67] (Fig. 10.2).

This method can regulate both the macroarchitecture (shape and size of the 3D object) as well as the microarchitecture (pore size, porosity, interconnectivity, etc.). The advantage of this technique as mentioned earlier is the simultaneous seeding of the cells facilitating uniform distribution and cell–cell contact leading to the formation of tissue like construct apart from its reproducibility [68,69]. However, the indispensable requirement for the polymer selected for 3D printing is that it should be able to either crosslink or undergo polymerization through UV light, laser, heat, or binder solutions to fabricate the scaffold. Physical crosslinking results due to noncovalent interactions like ionic, hydrogen bonds, which are weak in nature compared to the chemical crosslinking, which ensures a more stable 3D structure [70]. To enumerate few examples, gelatin, the denatured form of collagen, converts from sol to gel form at low temperatures thus facilitating cell viability. Apart from this, gelatin can also be chemically

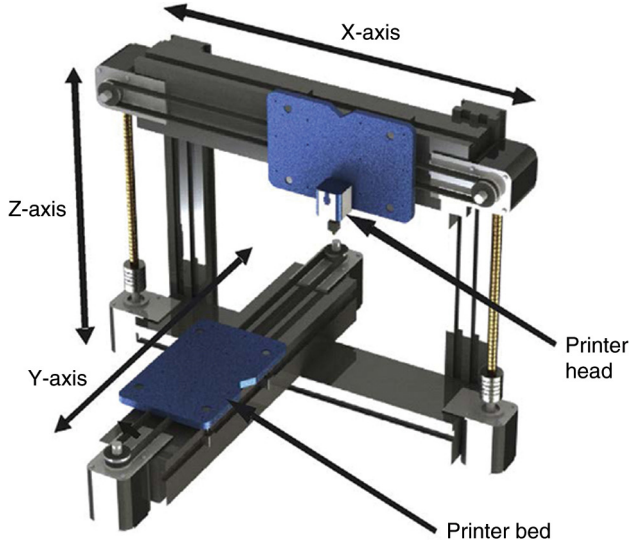

Figure 10.2 The image showing the XYZ axis of the 3D printer for proper positioning using printing bed and printer head (permission not required).

crosslinked in the presence of glutaraldehyde though cell viability has shown to be affected. Chitosan, obtained from crustacean animals, also forms a crosslinked gel in the presence of NaOH. Alginate can gelate in the presence of varying concentrations of $CaCl_2$ resulting in a crosslinked gel. Agarose solidifies at room temperature and thus form gels matrix. Hyaluronic acid upon exposure to light in the presence of photoinitiator can undergo crosslinking. Polyethylene glycol acrylates are also widely used for photopolymerization [71].

10.3.1 Direct 3D printing

1. Direct 3D printing is based on the convention 2D inkjet printer with the difference that the nozzle can print in "Z" axis also thus giving the construct a third dimension of height. Although the use of these techniques was initially limited to industrial applications, their ability to design myriad 3D shapes mend their ways into the field of biomedical and tissue engineering [72]. It is less time consuming compared to the other 3D scaffold synthesizing techniques as there is simultaneous seeding of scaffold, either with single type or multiple type of cells, in a controlled spatial arrangement during the 3D printing of the scaffold. Mainly three types of bioprinters are available for fabricating cell-laden hydrogel 3D tissue constructs.
2. Inkjet printers
   Ink jet printing is broadly classified into (1) Continuous-inkjet bioprinting, (2) electro-hydrodynamic jet bioprinting, and (iii) drop-on demand inkjet bioprinting of which drop-on demand method has gained enormous demand wherein the bioink is targeted to the specific location on a substrate using controlled volumes of ink essentially through thermal, piezoelectric, and mechanical systems. However, these printers suffer from limitations like cell viability due to usage of heat, frequency, and mechanical pressure, respectively. In addition, the nozzle clogging is also a major disadvantage in the above process [73–76] (Fig. 10.3).

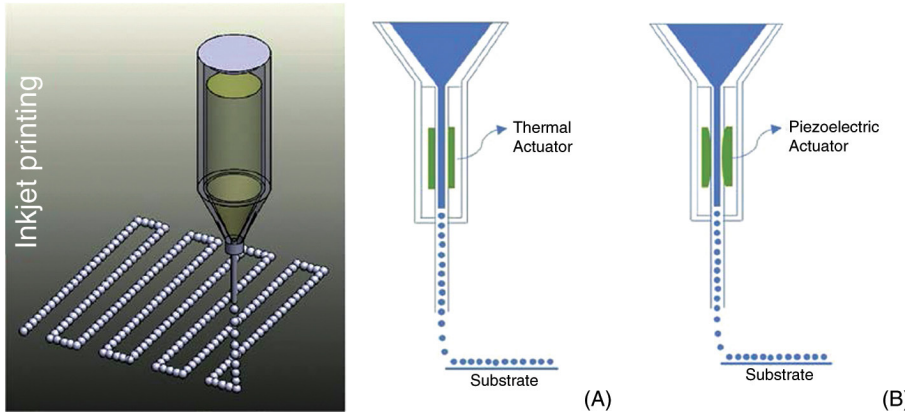

Figure 10.3 Schematic representation of drop-on-demand inkjet printing method by using (A) thermal actuators and (B) piezoelectric actuators. Printed under creative commons license from [74].

3. Microextrusion printers
   These printers use automatic robotic facility to dispense/extrude the bioink to design a 3D model. Primarily, the dispensing system is either pneumatic, piston, or screw driven. These extrusion-based systems have the advantage of using high viscous solution as bioink thus allowing usage of high concentration of cells without interfering with its resolution. Usage of low viscous solution is avoided as high-pressure during extrusion causes shear stress on cells and thus reduces the viability of the cells. Although alginate is biological inert and immensely used in the field of 3D printing by microextrusion, its nondegrading nature results in less proliferation and differentiation of seeded cells. However, combining it with gelatin and collagen in appropriate ratio resulted in the 3D printed construct, which had controllable degradation and showed increased proliferation and cell differentiation marker, cytokeratin, when cultured with human corneal epithelial cells compared to its control [77–79]. The bioink extruded should be such that it allows minimal resistance and undergoes physical or chemical crosslinking after extrusion to continue further process. This technique generates construct with high water content and therefore its use is mainly limited to soft tissue applications. However, the printer speed and resolution are few challenges that need to be overcome (Fig. 10.4).
4. Laser-assisted bioprinting (LAB)
   LAB uses focused laser beams to transform energy of the absorbing layer on the donor slide to generate high pressure such that it dispenses the cell containing material onto the collector slide. LAB is not a nozzle-based technique and therefore does not suffer from the disadvantage of nozzle clogging. As a result, the system can be used with material of varied viscosities. The limitation with this technique apart from being expensive, is the requirement of high gelation rate of the material used, the laser pulse and the metallic residues produced by the absorbing layer may cause deleterious effects on the 3D tissue. However, microextrusion and LAB allow use of high viscous material and thus cell aggregates and spheroids can be potential candidates for 3D tissue construct. Although it provides the most precise cell positioning, it lacks the vertical 3D construct formation and therefore is mostly applied in combination with other 3D techniques [80,81] (Fig. 10.5).

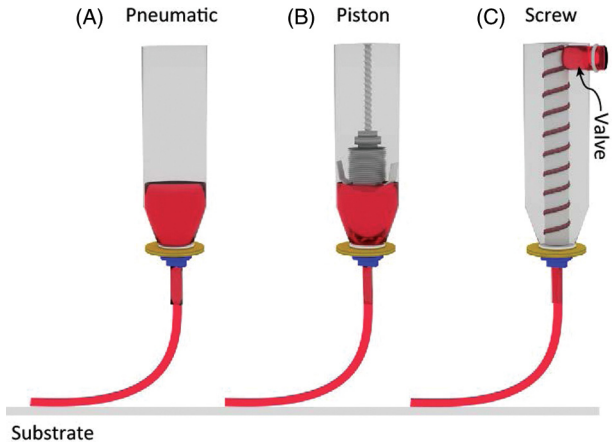

Figure 10.4 Schematic diagram of three types of extrusion-based bioprinting methods: (A) pneumatic, (B) piston-driven, and (C) screw-driven dispensing method. Published under the CC BY-NC-ND license from ref. [79].

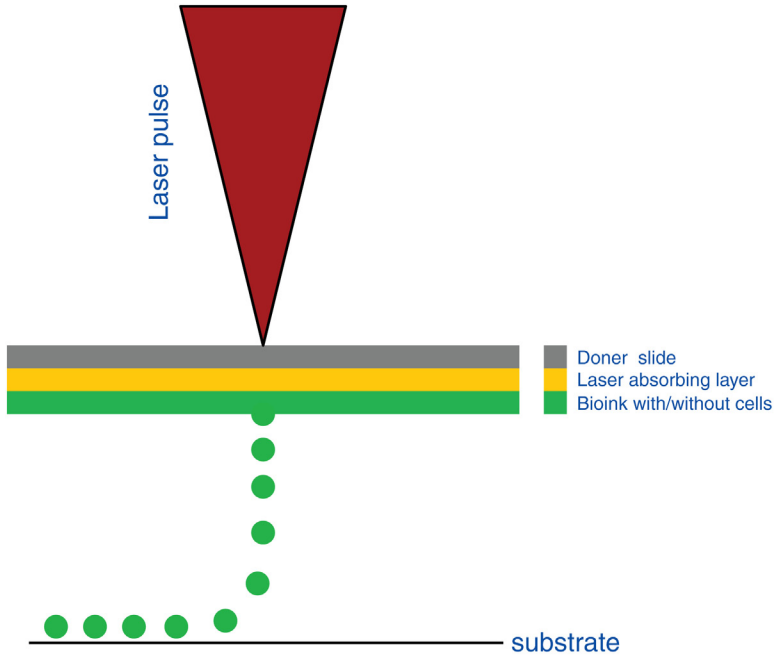

Figure 10.5 Schematic diagram of laser-assisted bioprinting. Pulsed laser source is used to deposit microdroplets of bioink on a substrate.
Source: Reproduce under creative commons license from ref. [74].

10.3.2 Fused deposition modeling (FDM)

FDM uses the low melting temperature property of thermoplastic materials to fabricate the 3D constructs. It uses two extrusion heads for the synthesis wherein one deposits the thermoplastic material and the other deposits the support material. The semiliquid material deposited on the build platform should be hot to allow rapid fusion with the initial extruded layer so that there is minimum flow. Although the synthesized scaffolds have high mechanical strength and high porosity, they lack the advantage of seeding cells or incorporation of bioactive molecules simultaneously due to high processing temperatures [24] (Fig. 10.6).

10.3.3 Selective laser sintering (SLS)

The powdered layer on the platform, instead of biopaper, is leveled using a roller system and then the binder solution is dispensed through the nozzle allowing the powder and the binder to combine. CO_2 laser beam is used instead in this technique. The build platform is let down and a new powdered layer is deposited. The above process is repeated to obtain the final 3D structure. The limitation of the technique is the improper removal of the binder solution after each step building up the toxicity levels. Moreover, the shrinkage of the material due to the use of laser and the post processing method of scaffold fabrication using heat (1400°C) is also an added disadvantage of the process [82]. SLS results in scaffold with high mechanical strength and low porosity, which are therefore immensely used for bone tissue engineering. The use of binder in direct 3D printing for synthesis of the scaffolds proves to be a limitation as it affects the viability of the seeded cells due to mostly its toxic nature. Therefore, another approach is used wherein the hydrogel is casted into a negative mold, which when dissolved leaves an intact 3D printed structure. The use of organic solvents also inhibits the use of aqueous printer heads with high resolution as they can be damaged. Lee et al. used water-based binder to 3D print the mold with calcium sulphate hemihydrate plaster powder, which was then casted with PLGA mixed with sucrose particles as porogen. The mold and porogen were then dissolved in an aqueous environment leaving an intact 3D printed scaffold [83] (Fig. 10.7).

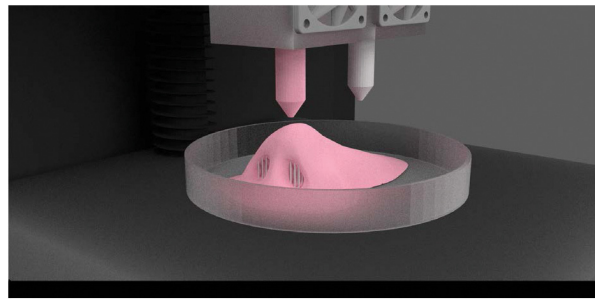

Figure 10.6 Dual head FDM 3D printer.
Source: Reprinted under open access from ref. [25].

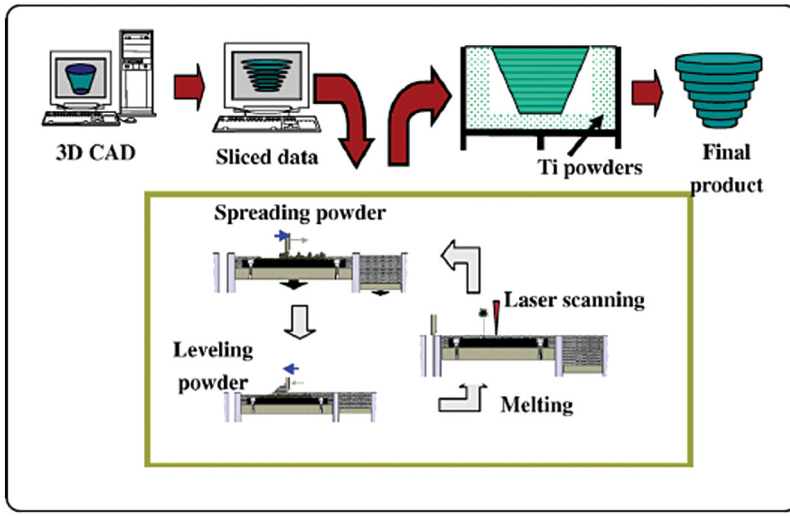

Figure 10.7 Schematic representation of selective laser sintering (SLS) technique. 3D scaffold is created by fusing together powdered particles using l laser.
Source: Reproduced with permission from ref. [84].

10.3.4 Stereolithography (SLA)

This technique uses light as a mediator for chemical reaction in the polymers for synthesis of scaffolds. The polymers used should be photosensitive and undergo cross-linking when exposed to the UV light. The first layer of polymer is cured after the deposition and then the process is repeated. SLA can use both the bottom-up approach as well as the top-down approach depending on which direction the cured layer is moved (either low or top) for the next uncured liquid resin to be patterned. The major advantage of the technique is its high resolution (1.2 μm) and the ability mimic the internal complex architecture of various shapes [84,85]. The major limitation is the unavailability of biocompatible resins and use of photopolymers and photo initiators, which releases radicals and unreacted monomers causing toxicity. Moreover, lack of mechanical strength and insufficiency in creating compositional gradient limits its use in the field of tissue engineering [86] (Fig. 10.8).

10.4 Decellularized ECM

3D bioprinting has emerged as a revolutionizing technology because it allows the precise and simultaneous seeding of the scaffold with the cells during its synthesis apart from being rapid, automated process. Moreover, single or mixed population of cells encapsulated in biomimetic hydrogels can be allocated precisely on the biopaper. The hydrogel provides various biochemical and mechanical cues to the cells allowing them to proliferate and differentiate. Studies have proven that decellularized extracellular material (dECM) can be used as an alternative to the various biomaterials

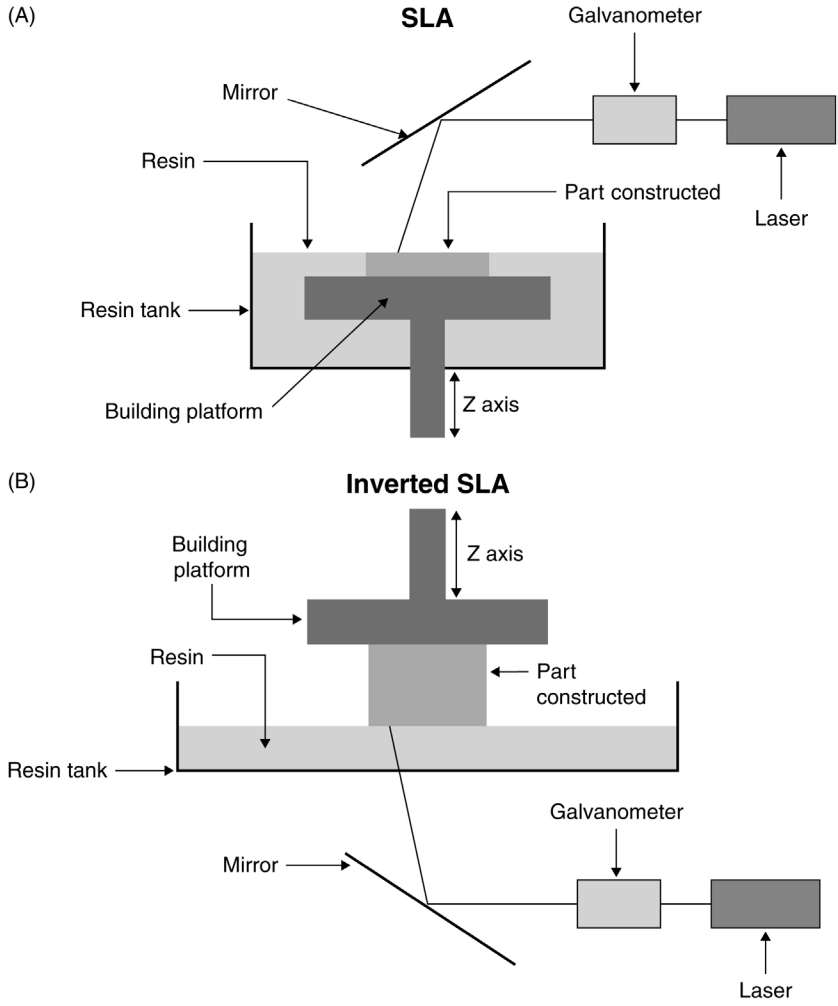

Figure 10.8 Schematic representation of stereolithography technique.
Source: Reproduced under open access from ref. [87].

used as it mimics the native microenvironment more closely and thus is gaining a lot of attention. dECM induces tissue remodeling and organization as the ultrastructure and composition of the tissue is preserved. In addition, it does not elicit any immune response, which is an indispensable requirement for long-term implantation studies. dECM, however, lack the required viscosity and mechanical strength and therefore it is used in combination with other biomaterials as blends/composites or as a supporting material as reported for tissue construct analogues [88,89]. However, reseeding of cells into specific compartment of the decellularized organ is still a challenge and various methods have been employed to overcome this problem. Vascular as well as nonvascular routes like trachea, ureter have been used as the pathways for the seeding

of the dECM compared to the injection at the various areas of the dECM [90–94]. Enhanced results have been obtained using bioreactor capable of perfusion as well as mechanical stimulation. With the advancement in the field, these matrices have also paved their way into the field of 3D printing. Although less viscosity of these matrices does lack the required mechanical strength but using it in combination with the other hydrogels have proved to be an alternative [95,96]. Skardal and coworkers used a two-step crosslinking procedure wherein gelatin and hyaluronic acid hydrogels were supplemented with dECM from liver, cardiac, and skeletal muscle thus resulting in bioinks with different stiffness [96].

10.5 Scaffold less approach

To overcome the limitation imposed by the use of hydrogels and their ability to bioprint in conditions compatible with cell viability, use of cell and cell aggregates in media has also gained momentum in the last few years.

10.5.1 Cell/cell aggregates

The principle of cell aggregate formation and their ability to mimic in vivo tissue is predominantly based on the studies of embryonic development where self-assembly of the cells of embryo and their differentiation into various tissues and organs occurs [97–101]. The advantage of using such cell aggregate is their inherent cell–cell contact and the presence of native microenvironment, which not only enhances the viability of the cells but also play an imperative role in cell proliferation and differentiation. The technique uses high cell density wherein either single cells encapsulated in hydrogel/media or cell aggregates in various forms like spheroids, cylinders, honeycomb, and torus are printed on a biocompatible substrate. Among all spheroids closely mimic the in vivo microenvironment and has been studied in detail. Time lapse studies using hepatoma cells have shown three stages leading to spheroid formation: (1) rapid cell aggregation, (2) delay period, and (3) tight compactness in the spheroid [102,103]. In addition, the high cell number decreases the time required for the formation of 3D microtissue and increases the cell survival of the 3D printed construct [104]. Cell aggregation is mainly the result of differential adhesion hypothesis (DAH), interfacial tension, and cytoskeletal contraction. Self-assembly and self-organization of the cells is predetermined by the type of cells and their preculture time. Studies have reported that longer preculture durations led to less compact tissue like structures than their counterparts because increased duration leads to more viscoelastic properties compared to their liquid state. Therefore, for the tissue construct to assume specific shape and integrity the fusion and maturation should be a rapid process. Thus, these cell aggregates can function as building blocks for the formation of tissue construct [104–110]. Conclusively, it has been reported that aggregation factor depends on various factors and can be calculated using the following formulae [111]:

$$Af = 1 - \frac{af}{n * ai}$$

Af = aggregation factor, *af* = sum of the areas of the faces of microgel, which is in contact with interface, *ai* = sum of the areas of one microgel faces, which is in contact with the interface, and *n* = total number of microgels.

Mammalian cells tend to show affinity toward specific cell types like the Chinese hamster ovary cells when grown in vitro embedded in hydrogel assumes a ring like structure. Moreover, it was reported that monodispersed NHF_s and $H35_S$ cells resulted in microtissue formation of various kinds by varying the preculture time. NHFs and H35s were fluorescently labelled with cell tracker red and green, respectively [71] (Fig. 10.9).

10.6 Biopaper

Cell printing to be successful and transform into formation of tissue/organ analogue structures, it is essential, for the printed cells to be motile, fuse, and maintain their viability. To achieve this, the substrate, should inhibit drying of the cells. Hydrogels,

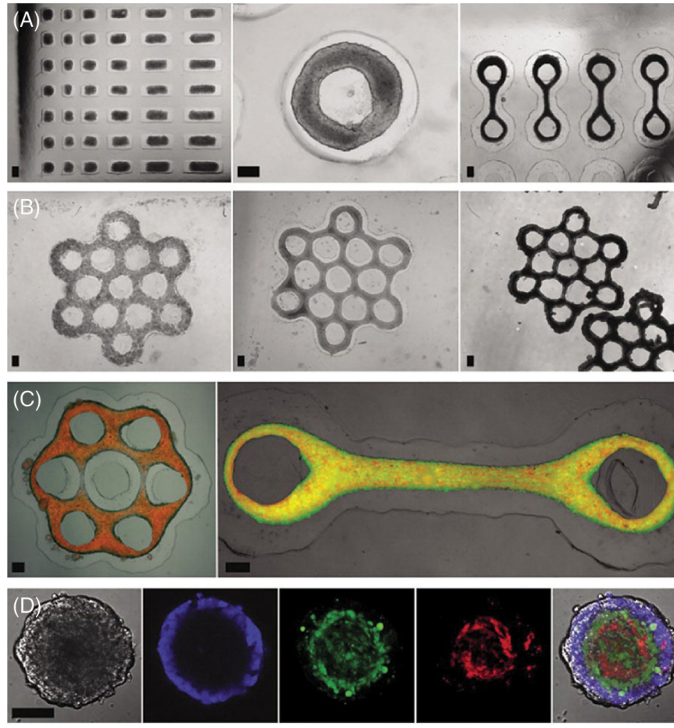

Figure 10.9 Microtissue size, shape, and composition are easily controlled in self-assemble cell–cell aggregates.
Source: Reprinted under creative commons license from ref. [112].

because of their capacity to retain water, has been reported to be the best solution [113]. Moreover, the biopaper should be soft to allow absorption of cell aggregates and maintain the optimum rate of cell motility so that the desired shape and structure is obtained instead of collapsing into its least state of energy, a large cellular aggregate. The biopaper should be such that it can be removed without disturbing the cellular structure once the cell fusion has occurred. The studies have reported that apart from the abovementioned feature, properties of the substrate gel, also plays a crucial role in fusion of cell aggregates. The interaction between the printed cell–biopaper and cell–cell decides the final pattern of the tissue [114].

10.7 Postprocessing

The functionality of the 3D printed constructs is mainly attained in the postprocessing process where the fusion of the cells/tissue occur in a bioreactor with predefined physiological conditions resulting in cell–cell contact and signaling. To effectively control tissue construct fusion and maturation, cell encapsulators, and automated biomonitoring systems, are available. Cell/tissue fusion is predominantly the result of surface tension forces, cell polarity, and cell cohesiveness. Union of a single type of cells is referred to as homotypic cell fusion compared to heterotypic cell fusion wherein different cell types merge thus resulting in the shrinkage of the whole 3D printed construct. Fast fusion in the study performed by Xu et al. [115] multiple cell types (human amniotic fluid-derived stem cells (hAFSCs), canine smooth muscle cells (dSMCs), bovine aortic endothelial cells (bECS) mixed individually with $CaCl_2$ crosslinkers were seeded on 3D printed pie- shaped composite of alginate collagen. During the postprocessing, the seeded cells fused and formed functional tissues in mice with 99% cell viability [116]. Since the 3D printed tissue analogue undergoes change in its shape during postprocessing in a bioreactor, a fourth dimension, time period is also added making it sometimes referred to as 4D printing instead of 3D printing (Fig. 10.10).

10.8 Tissue formation

Tissue liquidity and differential adhesion hypothesis forms the basis for the heterotypic/homotypic tissue formation among which the “sphere” formation is studied to be the least energy state [111]. Studies with NHF microtissues showed 7 days of preculture time resulted in rod-like structure, unlike the spherical structure adopted by monodispersed cells. Further microtissues showed linear rate of contraction compared to monodispersed culture, which adopted an exponential rate. Although tissue stability is inversely proportional to their surface area, this mathematical model has been shown to be insignificant when tissue fusion is compared in large and small microtissues. However, microtissue maturity has been observed to play a significant role. Preculture time, cytoskeletal mediated cell kinetics have also been an indispensable factor in cell sorting and positioning in tissue formation [118,119]. Cell fusion/coalescence

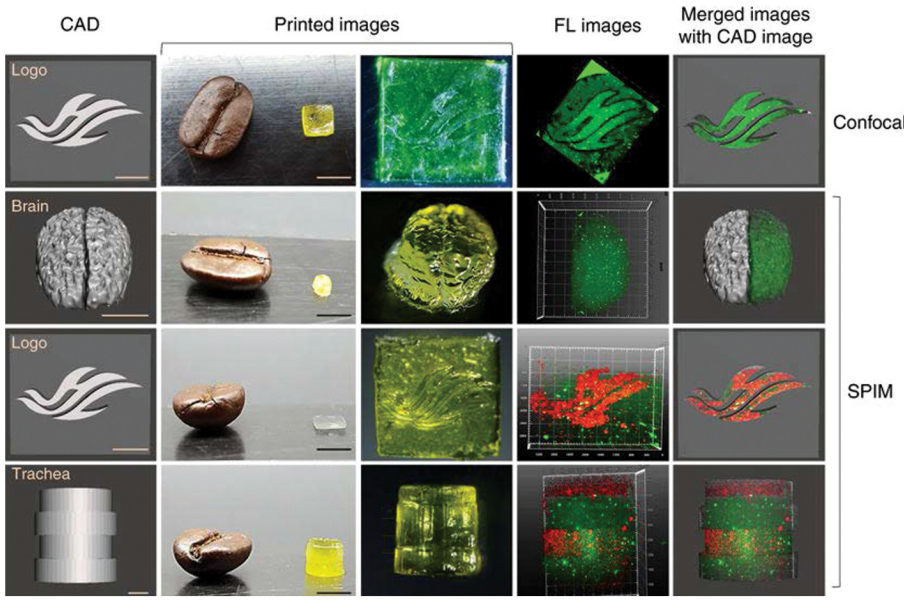

Figure 10.10 Schematic representation of bioprinting using cells and hydrogel material as bioink, which further merges to form 3D structure. Cell and material interaction define the success of postprocessing printed material.
Source: Reproduced under open access from ref. [117].

is essentially demixing of a population of cells into different phases resulting in cell sorting whereas at the tissue fusion is primarily based on cell–cell or/and cell–ECM interaction, their proliferation and ECM production [120]. This self-assembly is also mediated by thermodynamic and surface tension of the liquid air interface wherein cell-laden hydrophilic hydrogel (low density) forms aggregates to achieve a more stable structure with minimum free energy. These were then chemically crosslinked to achieve stable centimeter scale tissue constructs. It has been reported by Jens et al. that microtissues produced using monodispersed cells like C_2C_{12} (myofibroblasts), chondrocytes from pig and human have successfully resulted in macrotissue patches of mm^3 scale and particular shape. Mediolateral intercalation, which results in elongation in 2D and 3D structures results in concomitant decrease in their width or diameter.

10.9 Bioactive molecules

3D printing technique has also been successfully in using various biomolecules as bioink. Various angiogenic factors, growth promoting factors, and chemical regulators have been loaded on the substrate in desired concentration and location. Studies have shown that either the stem cells are differentiated in vitro and then assembled layer-by-layer for the tissue construct or the homogenous layer of stem cells are

assembled with various growth factors, ECM and regulators sequentially to enable them to differentiate into the desired cell population on the 3D printed construct. In order to achieve appropriate cell–cell, cell–ECM, and cell-growth factors association the homogenous layer of cells should be approximately 20 μm or less and it requires further improvement and accuracy in the delivery techniques [26,121,122].

10.10 Vascularization

The basic requirement for a tissue to be functional is proper diffusion of nutrients and oxygen and therefore any tissue which surpasses thickness of 150–200 μm is deficit in the proper exchange and thus is unable to survive for longer duration. In the conventional techniques, this poses a severe limitation on the thickness of the synthesized scaffold. However, with 3D printing and its ability to simultaneously seed the scaffolds with combination of cell types (using multiheaded printer), it is possible to simultaneously synthesize mini-vasculature in the 3D printed tissue by concurrently using endothelial cells and the desired cells as separate bioink [123]. Vascular tubular constructs of 300–500 μm were constructed using agarose cylinders as template molds and spheroids or cylinders of smooth muscle cells and fibroblasts layer-by-layer. The engineered blood vessel was synthesized in different shapes and diameters using agarose rods as templates [124]. Ground breaking research by L'Heureux has led to the construction of vascular grafts, which are currently under clinical trials. The group used the principle of self-assembly in designing sheets of smooth muscle cells and fibroblasts in vitro which was then detached and meticulously wrapped around a tubular mandrel. After maturation in a bioreactor, the lumen of the construct was seeded with endothelial cells resulting in multi-layer well organized vascular graft [125].

10.11 Liver

Liver plays a vital role in metabolism regulation. The hepatic parenchymal cells comprise approximately 78% of the liver and is primarily involved in the metabolic functions. Various 2D and 3D liver models, using parenchymal spheroids, have been proposed since the conception of the idea of tissue engineering. However, none have been able to maintain the dynamic function of the liver for long duration. In the article published by Kizawa et al., scaffold-free small portion of 3D liver-like tissue was printed, which could maintain its functionality (glucose, drug and lipid metabolism) for a period of 75 days of culture [124]. In addition the expression level of various transporters and enzymes were significantly high compared to 0 day hepatocytes. The model could also be potentially used for drug metabolism studies and hepatotoxicity which would pave way for medical treatments [126] (Fig. 10.11).

3D liver models were constructed using stereolithography, which served as preoperative models for surgery in addition to studying its anatomical details and surgical risks. The model served as a prospective candidate for the study of the tumor and its characteristics, which proved supportive in pediatrics liver surgery (Fig. 10.12).

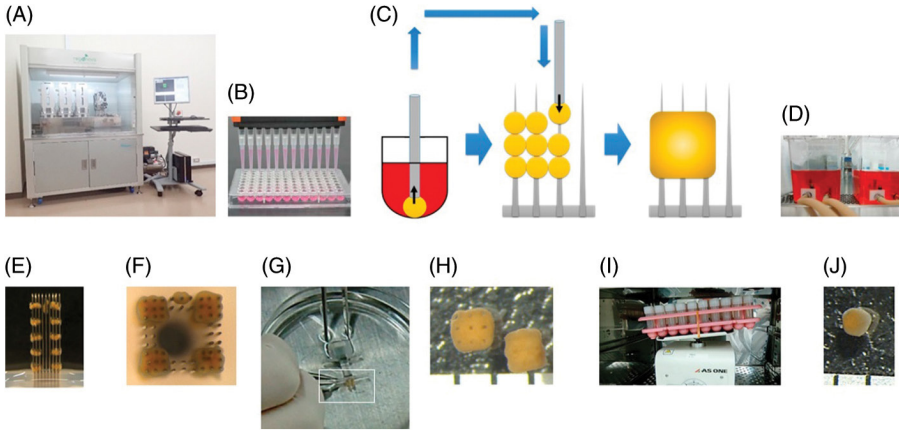

Figure 10.11 3D printing method for generating human liver tissue. (A) Photograph of the used Regenova apparatus. (B) Photograph of spheroids cultured in 96-well plate. (C) Schematic representation showing (left) collection of spheroid, (middle) then placed on skewers, and (right) finally tissue formation due to their fusion on the needle array. (D) Photographic image of the perfusion chambers for spheroids skewered onto the needle array. Photographs showing the (E) side view and (F) top view of bioprinted liver tissues. (G) Photograph showing the retrieval of the bioprinted liver tissue. (H) Photograph of bioprinted liver tissues. Bioprinted cubical tissue with nine holes created by the 9 × 9 needle array. Scale = 1 mm. (I) Photographic image of rocking cultures for sustaining printed liver tissues. (J) The spherical bio-printed liver tissue at the end of 60 days with a diameter of ~1 mm. Scale = 1 mm.
Source: Reproduced with copyright from ref. [124].

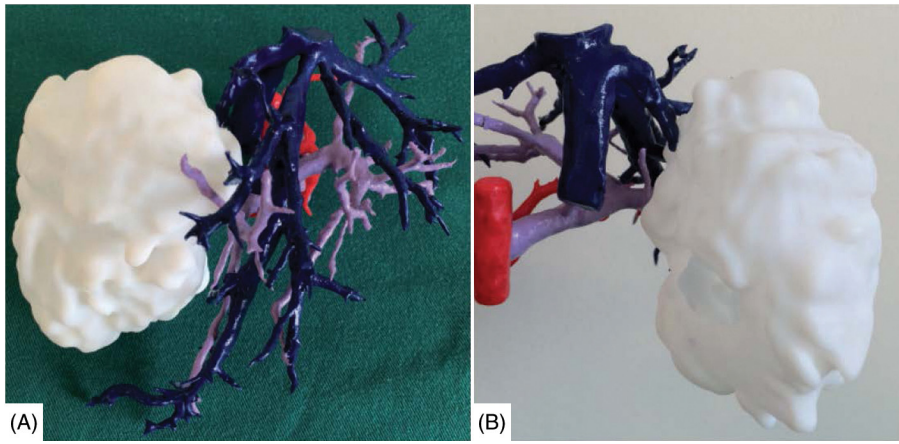

Figure 10.12 Three-dimensional (3D) representation of a printed model of a hepatic tumor in a child: (A) anterior view and (B) posterior view. The tumor is represented in white, the hepatic vein is shown in blue, the portal vein is in purple, and the artery is red. The data from computed tomography were converted to .stl format files, which were then electronically delivered to a 3D printer to be printed with photosensitive resin.
Source: Reproduced under creative common license 4.0 from ref. [125].

10.12 Skin

Gelatin impregnated with sulfonated silk fibroin was used by Xiong et al. as the 3D printed scaffold for skin tissue regeneration. The construct, unlike the scaffolds synthesized by the conventional methods, were capable of skin regeneration in full thickness defect model in the rat. Further the scaffold was also incorporated with FGF-2 known to stimulate cell proliferation and migration. Sustained release of the growth factor showed improved tissue morphology, blood vessel formation, collagen fibrils, and marker expression. Apart from this the microscale architecture of the 3D printed construct stimulated reepithelialization and dermal vasculature thus proving it to be a viable therapeutic strategy for skin defects [127] (Fig. 10.13).

3D printed dermal equivalents were also printed using a PEG-based bioink, which polymerizes upon illumination at 365 nm to form a crosslinked hydrogel. The 3D model was constructed such that it had alternate layers of abovementioned polymer and fibroblasts layer. The formed construct was topped with a ring of seven cylindricals, which were later seeded with keratinocytes to mimic the epidermal layer [124]. The whole 3D printing process took 7 min and resulted in highly reproducible and stable construct (Figs. 10.14 and 10.15).

Recent study by He et al. has advanced the technology of 3D printing in skin tissue engineering by in situ printing wherein the construct is synthesized precisely at the wound site in the rat using pressure driven printing system [127]. Amniotic fluid-derived stem cells and mesenchymal stem cells were seeded layer-by-layer alternating with thrombin layer which acts as the crosslinking agent. The 3D printed model

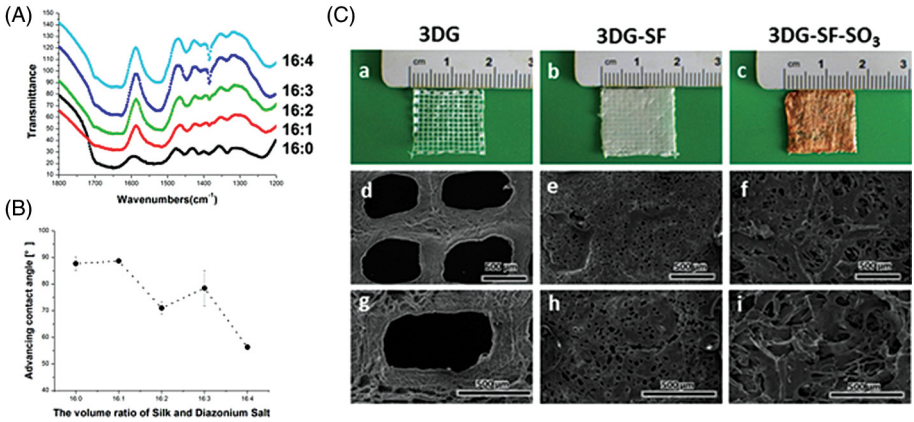

Figure 10.13 Characterization of scaffolds. (A) Photograph of the 3D printed (3DG) scaffold, (B) silk fibroin (SF)-coated 3D printed scaffolds (3DG-SF), and (C) sulfonated SF coating the 3D printed scaffold (3DG-SF-SO_3). Scanning electron microscopy (SEM) images of 3DG scaffold (D), 3DG-SF scaffold (E), and 3DG-SF-SO_3 scaffold (F) with (g, h, i) at high magnification. Each layer is 100 μm thick leading to the whole thickness of bio printed gelatin and gelatin coated with silk fibroin derivative scaffold to be 1 mm. Scale bars, 500 μm. *Source*: Reprinted under open access from ref. [126].

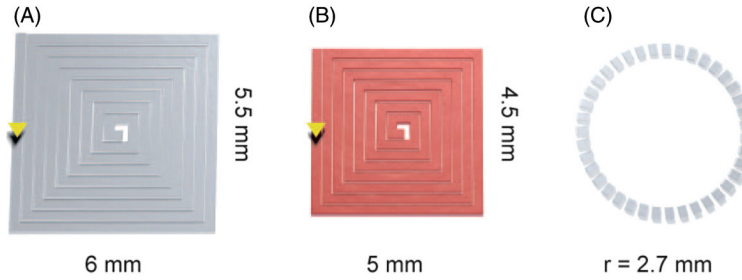

Figure 10.14 Schematic representation of the 3D dermal grafts by using layer by layer mode. The bioink solution was printed in the dimension of (A) 6 × 5.5 mm in an angular spiral pattern (B) similarly fibroblast suspension was printed in 5 × 4.5 mm dimension. The yellow arrow in the figure is indicative of the printing direction. (C) A cylindrical structure comprising of seven rings, each ring being 2.7 mm in radius, was bioprinted on top of this dermal layer to serve as the substrate for keratinocyte seeding.
Source: Reprinted under open access from ref. [128].

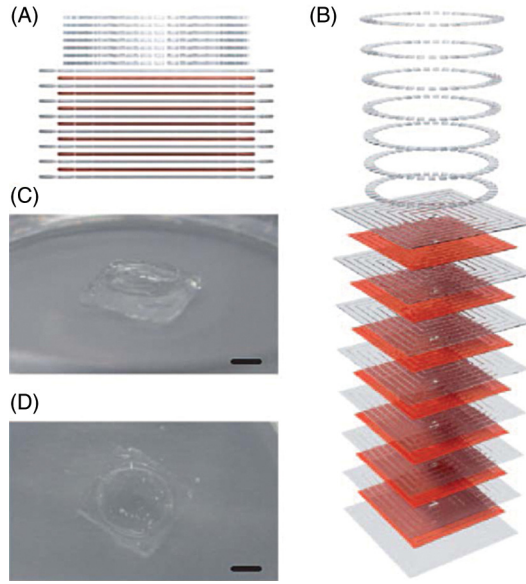

Figure 10.15 Schematic representation of printed 3D dermal models. (A) Side view of the dermal equivalent is shown in the figure with alternating bioink spiral layers (gray lines) and seven fibroblast suspension spiral layers (orange lines). This dermal equivalent is capped with seven circular bioink layers (gray dotted lines) forming a cylinder. (B) The 3D printed dermal equivalent is clearly represented with alternating layers of bioink and cell suspension topped with cylinder. Photograph of the printed dermal equivalents wherein the cylindrical structure is visible. Scale bar in (C) and (D): 2 mm.
Source: Reproduced under open access from ref. [128].

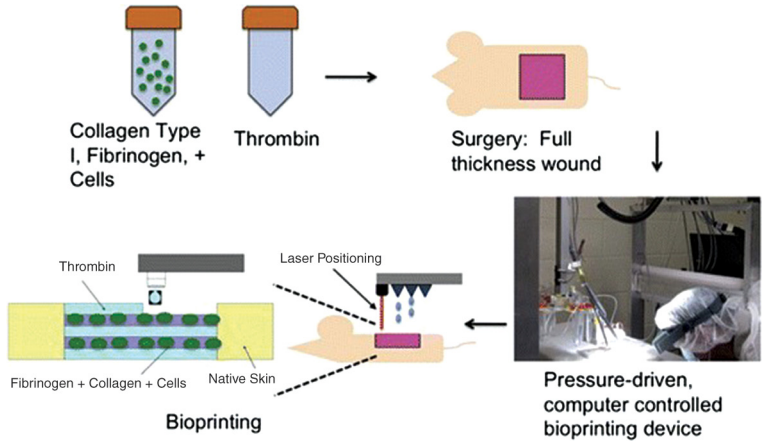

Figure 10.16 Schematic representation showing in situ bioprinting.
Source: Reprinted under open access from ref. [96].

showed enhanced re-epithelialization and wound closure. Although this paves a way for highly precise and advanced synthesis of scaffolds it is posed with major limitations like sterility, cell localization, etc. (Fig. 10.16).

10.13 Conclusion

3D printing technology has enabled almost full control over the design and fabrication of in vivo tissue and organs by closely resembling the hierarchical architecture. It is a rapid, inexpensive procedure and provides a new spectrum in the field of tissue engineering. Tissue formation in the presence or absence of scaffolds has also opened a new avenue. Cell/tissue fusion and their further postprocessing has led to generation of optimum 3D scaffolds.

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Transplantable scaffolds

11

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Chapter outline

11.1 Introduction

Tissue engineering (TE) is a multidisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes to restore, maintain, or improve tissue function [1]. TE involves the use of living cells to develop biological substitutes for implantation into the body and/or to foster the remodeling of tissue in some other active manner [2].

Two main approaches are used in the field of tissue engineering. The first one is the use of scaffolds as a cell support and template to encourage the cells to lay down their own matrix. The scaffolds can also be used as growth factor/drug delivery devices to induce/aid regeneration inside the body [3]. The second one is a scaffold-free approach, which has its origins in self-assembly. This strategy involves the use of prefabricated multicellular building blocks such as cell sheets and spheroids [4]. Fig. 11.1

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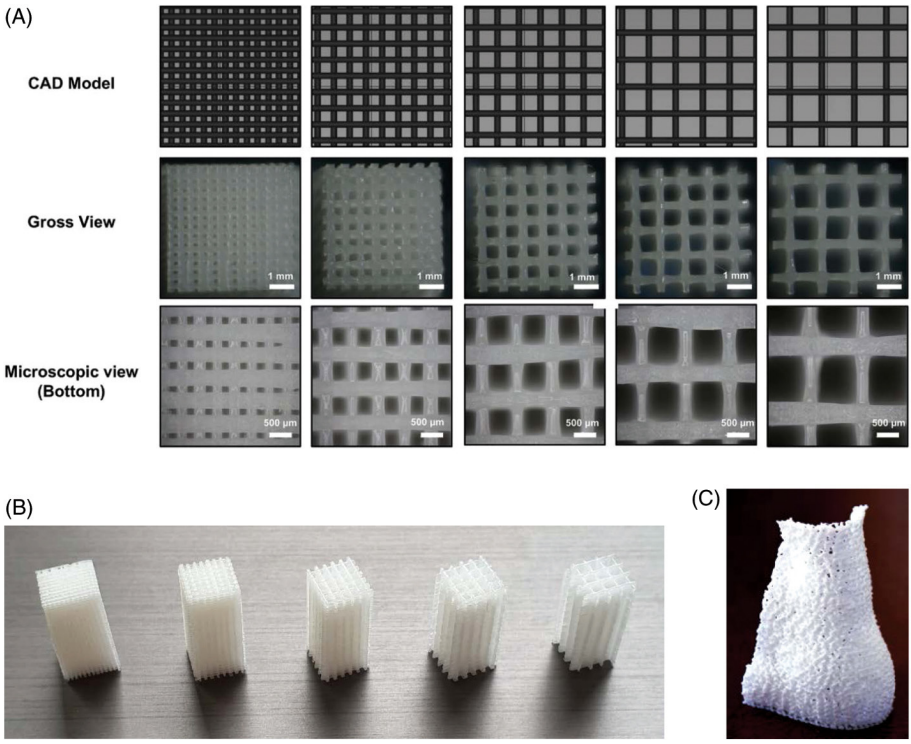

Figure 11.1 Cuboidal PCL scaffolds with five different porosities, which were manufactured by material extrusion additive manufacturing and used for cell culture studies (A) and (B). Example of a scaffold with anatomical geometry of a nose (C).
Source: Section (A) is adapted with permission from [16].

shows example scaffolds used for cell culture (cuboid specimens) and to reproduce anatomical geometry (human nose).

TE relies extensively on the use of porous scaffolds to provide the appropriate environment for tissue regeneration. Numerous scaffolds have been produced from a variety of biomaterials and different manufacturing processes in attempts to target the regeneration of different tissues [5]. Regardless the tissue type, there are four key elements that the scaffolds should have. Firstly, it should be able to mimic the architecture of the native extracellular matrix (ECM) by providing space for vascularization, new tissue formation, and nutrient transport. Secondly, the scaffold should interact with the cellular component to facilitate their activities such as proliferation and differentiation. Thirdly, the scaffold has to provide a 3D structural support while matching the mechanical properties of native tissues/organs [6]. Lastly, they should preferably be biodegradable and the degradation bi-products should not be toxic and able to exit the body without damaging other organs [7].

Since a major purpose of tissue engineering scaffolds is to provide a mechanical structure for cells to grow on or within, mechanical properties are critically important.

The mechanical requirements may range from very low (e.g., to support the weight of hydrogel) to very high (e.g., to shield a healing bone from excessive mechanical loading during bone fracture repair or new bone growth). The choice of scaffold material is potentially the most important aspect with regards to mechanical properties since scaffolds may be produced with materials ranging from flexible, such as polycaprolactone, to rigid, such as bioactive glasses. In addition to the material choice, the structural geometry of the scaffold plays an important role in mechanical properties [8]. In particular, porosity is known to strongly affect mechanical properties [9–15], as does the design and orientation of lattice struts [8], among other factors.

Additive manufacturing is commonly used to manufacture scaffolds because of its ability to create porous structures with complex internal geometries. There are numerous different categories of additive manufacturing processes, ranging from laser-based systems that melt metal power particles together to syringe-based systems that extrude cell-laden hydrogels. All additive manufacturing technologies share a common ability to selectively position or bond material in order to generate a 3D part in an incremental manner. In contrast, subtractive processes incrementally remove material from a larger initial solid volume. Hence, it is difficult for subtractive processes to navigate within complex internal pores/geometries (e.g., it is not possible to drill a hole that changes direction partway along its length).

The scope of this chapter is to predominantly consider scaffolds manufactured by material extrusion additive manufacturing, which is also known as extrusion 3D printing or often referred to as bioprinting within the medical field. In this rest of this chapter, an overview of common additive manufacturing processes for scaffolds is given in Section 11.2 followed by a discussion of common materials used for scaffolds in Section 11.3. Section 11.4 discusses the mechanical properties of scaffolds. Methods of seeding of scaffolds with living cells and cell proliferation are presented in Section 11.5, followed by analysis of longer-term performance of scaffolds for tissue maturation in Section 11.6. Finally, a conclusion and future outlook is given.

11.2 Scaffold manufacturing processes

Scaffolds have been manufactured in a huge variety of ways, which have been reviewed in a number of review articles [17–20]. The review article of Turnbell et al. [18] gives a good summary of the conventional manufacturing methods, which typically manufacture scaffolds via the generation of pores within a bulk material. These include solvent casting, particle leaching, gas foaming, emulsification, freeze drying, and phase separation [18].

Over the last two decades, additive manufacturing technologies have become popular for the production of scaffolds because they can add material with microscale precision at specific positions to generate a porous 3D structure. Schematics of four common additive manufacturing processes are shown in Fig. 11.2: material extrusion, binder jetting, stereolithography, and powder bed fusion. These processes will be discussed briefly here, but more information can be found elsewhere [17,21,22].

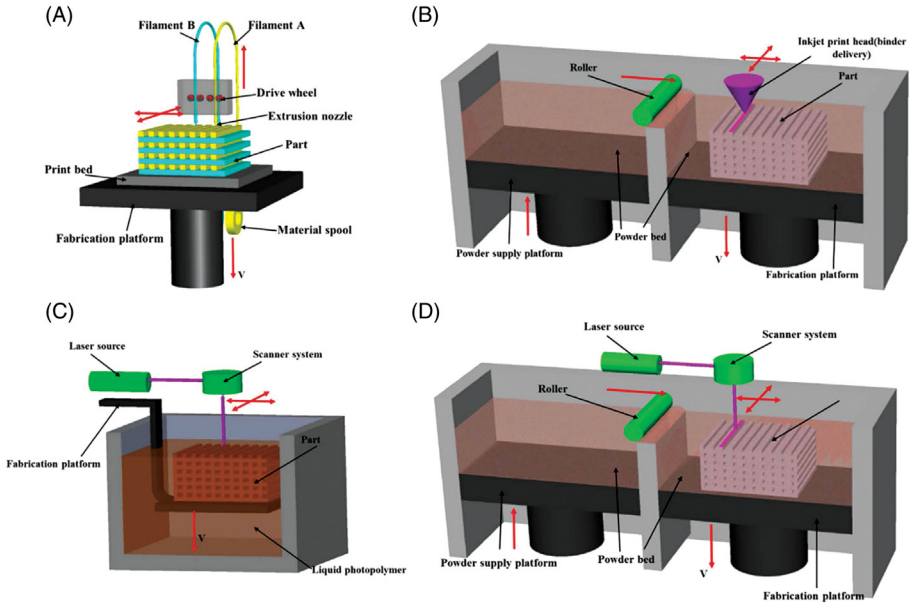

Figure 11.2 Four categories of additive manufacturing processes: (A) material extrusion, (B) binder jetting, (C) stereolithography, and (D) powder bed fusion.
Source: Reproduced with permission from [17].

In material extrusion additive manufacturing (Fig. 11.2A), also referred to as fused deposition modelling (FDM) or fused filament fabrication (FFF), the product is fabricated by extruding material from a nozzle at specific locations, similar to the operation of a hot-melt glue gun. The 3D object is built up by depositing material in a layer-by-layer manner, analogous to a log cabin being made by stacking logs on top of each other (where the logs represent filaments extruded from the 3D printer nozzle). In binder jetting (Fig. 11.2B), powder particles are spread into a flat bed and then a binding material (e.g. a resin that cures to solid form) is jetted into the powder at specific locations; the powder is only bonded together at the positions where the binder is jetted. After completing one layer, a new layer of powder is spread on top of the previous layers and the jetting process is repeated. This layerwise addition of powder and binding material ultimately results in a 3D structure. In stereolithography (Fig. 11.2C), a laser is used to cure (solidify) resin at specific locations, before a new layer of resin is spread on top of the existing layer and the laser-curing process is repeated. This requires the material to be photocurable. In powder bed fusion (Fig. 11.2D), a similar process to binder jetting is used, except the powder particles are melted or sintered together with a laser rather than bonding with a binder material. Some advantages and disadvantages of each process are indicated in Table 11.1. Other additive manufacturing processes, along with variants of those described above, are described in dedicated review papers [17–20].

Table 11.1 Summary of several additive manufacturing processes.

Technique	State of starting materials	Typical polymer materials	Working principle	Resolution (Z direction, μm	Advantages	Disadvantages
FDM	Filament	Thermoplastics, such as PC, ABS, PLA, and nylon	Extrusion and deposition	50-200 (Rapide Lite 500	Low cost, good strength, multi-material capability	Anisotrophy, nozzle clogging)
SLA	Liquid photopolymer	Photocurable resin (epoxy or acrylate based resin)	Laser scannin and UV induced curing	10 (DWSLABXFAB)	High printing resolution	Material limitation, cytotoxicity, high cost
SLS	Powder	PCL and polyamide powder	laser scanning and heat induced sintering	80 (Spo230HS)	Good strength, easy removal of support powder	High cost, powdery surface
3DP	Powder	Any materials can be supplied as powder, binder needed	Drop-on-demand binder printing	100–250 (Plan B, Ytec3D)	Low cost, multimaterial capability, easy removal of support powder	Clogging of binder jet, binder contamination
3D	Liquid or plotting paste	PCL, PLA, hydrogel	Pressurized syringe extrusion, and heat or UV-assisted curing	5-200 (Fab Home)	High printing resolution, soft materials capability	Low mechanical strength, slow

FDM (fused deposition modelling) and 3D plotting are variants of the material extrusion additive manufacturing process. SLA = stereolithography. SLS = selective laser sintering, also known as powder bed fusion. 3DP = 3D printing, also known as binder jetting.
Reproduced with permission from [17].

Material extrusion additive manufacturing is the main focus of this chapter due to its popularity in the tissue engineering field. This popularity arises from its capabilities to process a wide range of materials and to print multimaterial structures, the simplicity of operation (with relatively low requirements for auxiliary equipment) and potential for relatively low costs. There are several variants of material extrusion additive manufacturing, as shown in Fig. 11.3. The most common variant uses a filament reel of raw polymer (typical diameter 1.75–3 mm), which is fed into the top of a heated chamber and extruded out of a nozzle on the underside (nozzle diameter typically 0.1–0.5 mm), as shown in Fig. 11.3A. The rate of material extrusion is controlled by how quickly the material is fed into the heated chamber. The main drawback of

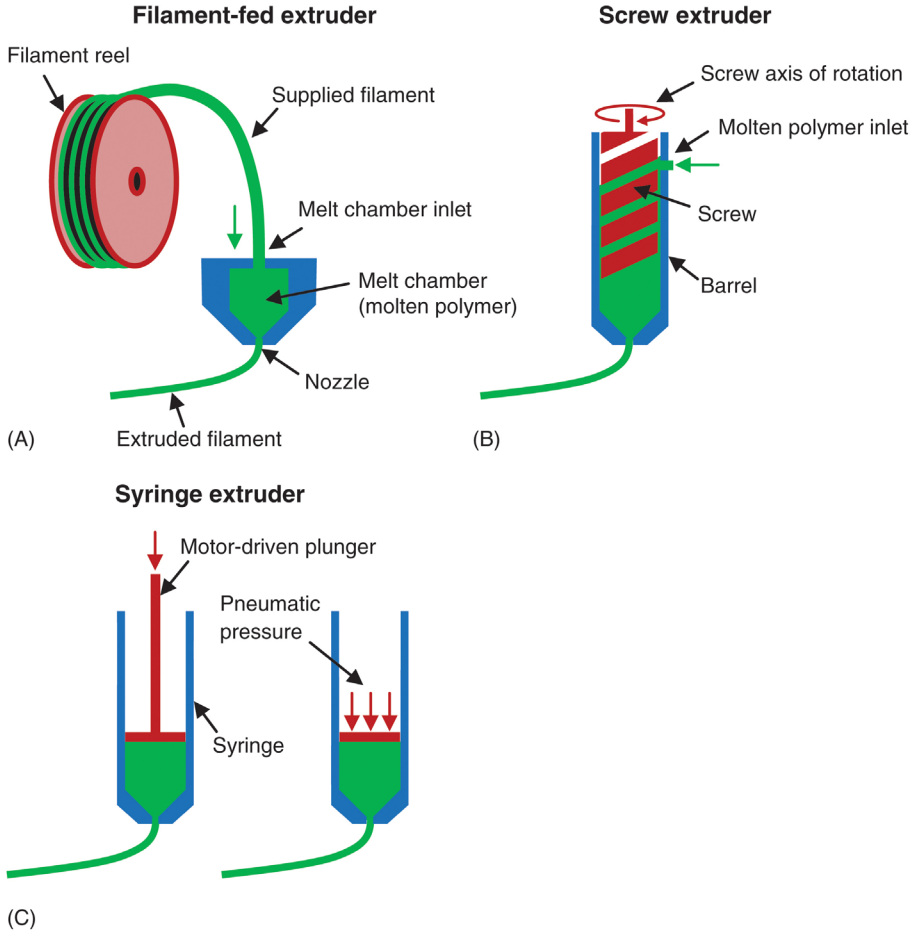

Figure 11.3 Three variants of additive manufacturing extrusion technologies: (A) filament-fed, (B) screw-extrusion, and (C) syringe extruders driven by a motor-driven plunger or pneumatic pressure.
Source: Reproduced with permission from [8].

this approach is that the input material must be in filament form. To allow pastes and hydrogels to be used, a barrel or syringe may be implemented in the 3D printer. The paste or hydrogel is loaded into the syringe (or a connected hopper); it is then forced out of the syringe by a screw mechanism, similar to widespread industrial extrusion processes (Fig. 11.3B), or a plunger (Fig. 11.3C), which can be controlled by pneumatic pressure or a motor.

The term "bioprinting" does not have an explicit definition but bioprinters often take the form of a material extrusion additive manufacturing system that includes several printheads and therefore allows multiple materials to be printed in a single structure. This allows multiple structural scaffold materials to be used (e.g. to achieve graded mechanical properties), multiple different cell-laden hydrogels to be used (e.g. to spatially distribute different cell types for printing complex tissues/organs), and many more innovative uses.

3D printer software is a critically important aspect of the overall manufacturing process. Most 3D printers come with their own software, and some open source software packages are available, such as Cura [23] or Slic3r [24], but these have been developed for conventional engineering applications and are typically not well suited to bioprinting. The main purpose of 3D printing software is to generate a print path—this is the path that the nozzle follows to deposit material in a particular pattern/geometry. The user is not typically responsible for designing the print path; instead, they load a 3D model of the desired overall external geometry into the 3D printer software and it automatically generates a print path to produce a structure with the geometry of the 3D model. For scaffolds, the structure is required to be porous as opposed to solid, so the print path must allow gaps between each individually extruded filaments The software packages offer the user the ability to control some aspects of the printing process, but for intricate scaffolds structures it may be necessary to use in house software that allows full control over the print path [16,25,26]. Further details about 3D printer software can be found in a recent review paper [8].

11.3 Scaffold materials

There is a huge range of materials that can be used for scaffolds. They can be biodegradable or permanent, natural or synthetic, and can often be combined in a mixture or composite to integrate desirable properties from different materials. The range of materials, for each of which the advantages and disadvantages will be discussed in the following paragraphs, includes:

- Hydrogels and biological materials such as alginate [27–29], fibrin [30], collagen [31,32], gelatin [33,34], and polyacrylamide [35].
- Biodegradable polymers such as biodegradable polylactide, polyglycolide, polycaprolactone, or blend/copolymer mixtures of these polymers [36,37].
- Nonbiodegradable polymers such as polyetheretherketone [22], polymethyl methacrylate [38], and polyethylene glycol [37].
- Ceramics and glasses such as hydroxyapatite, tricalcium phosphate and silicate, or phosphate glasses [36].

- Metals such as tantalum, magnesium, stainless steel, titanium, and many alloys of multiple metals [20].
- Composites and multimaterial scaffolds such as fiber-reinforced polymer scaffolds [19] or combined polymer and hydrogel scaffolds [25].

11.3.1 Hydrogels and biological materials

Hydrogels are commonly used in tissue engineering due to their low toxicity and structural similarity to ECM [39]. Their highly hydrated network permits exchange of nutrients and gases and makes them an attractive option for the formulation of bioinks. An ideal bioink should satisfy the following properties: printability, biocompatibility, and good mechanical properties to retain structural stability after printing [40]. Printability means that hydrogels must be suitable for extrusion deposition, with adequate viscosity, shear thinning properties, and short response crosslinking time [41]. The physicochemical properties of a hydrogel that determine their printability are its rheological properties and crosslinking process [42]. The hydrogel should be viscous enough to be dispensed as a free-standing filament. However, if the gel is too viscous, large forces are required to extrude the bioink resulting in cell death [42]. In terms of biocompatibility, the hydrogels should have an appropriate degradation time, be able to support cell attachment and not cause immune response. Other important characteristics that need to be considered is the mechanical strength after crosslinking [43].

Hydrogels can be natural (biological materials), synthetic, or combinations of both. Some of the natural hydrogels are alginate [27–29], fibrin [30], collagen [31,32], hyaluronan [44], chitosan [45], gelatin [33,34], Matrigel [46], and gelatin methacrylate (GelMA) [47]. Examples of synthetic hydrogels are polyethylene glycol diacrylate (PEGDA) [48], polymethacrylates (PMA), and polyacrylamide (PAM) [35]. Biological materials are either proteins sources or polysaccharides. The main advantage of these natural materials is that they can interact with the cells due to their interactions with cell surface receptors; allowing cell migration, proliferation, and ECM production [49]. In some cases, these interactions can be a disadvantage because these polymers may also stimulate an immune response and be subject to very fast biological degradation processes. Additionally, these materials also suffer from batch to batch variability [50].

Alginate is a polysaccharide extracted from brown algae, and is widely used for cartilage tissue engineering, chondrocytes cell expansion, and redifferentiation [51–53]. The main benefits are biocompatibility, ambient gelling conditions, and ability to maintain chondrocytes phenotype when expanded in vitro [54].

Chitosan is also a polysaccharide; it is derived from the natural polymer chitin via partial deacetylation. The major advantage of chitosan is that its physicochemical and biological characteristics can be highly tailored by utilizing the reactivity of glucosamine residues. Like other naturally derived polysaccharides, chitosan is typically combined with other materials such as polycaprolactone to enhance its properties in cartilage repair [55].

Fibrin is a fibrous protein mainly responsible for the formation of blood clots. Fibrin hydrogels are easily fabricated by crosslinking fibrinogen. In vivo animal studies have shown that the combination of autologous chondrocytes and allogenic devital-

ized cartilage matrices suspended in fibrin glue allows the formation of cartilage-like tissue [56].

Collagen is the most abundant protein present in the cartilage ECM. This protein is able to polymerize into a stable gel at neutral pH and physiological temperatures. In addition, collagen hydrogels have good cell adhesion properties.

Gelatin exists as a mixture of water-soluble protein fragments, comprised of the same amino acid sequences as collagen, from which it is derived. Importantly, the bioactive sequences of collagen for cell attachment and matrix metalloproteinase (MMP)-sensitive degradation sites are retained in the gelatin backbone [57]. As such, essential cellular functions, such as migration, proliferation, and differentiation, can be facilitated via integrin-mediated cell adhesion and cell-mediated enzymatic degradation [58].

11.3.2 Polymers

Polymers have been used for clinical applications for several decades. A critical distinction is between biodegradable and nonbiodegradable polymers: biodegradable polymers are absorbed into the body over several weeks, months, or years, depending on the polymer and its molecular weight; nonbiodegradable polymers ideally maintain their form and mechanical integrity over a nominally permanent lifetime. Common biodegradable polymers include polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), polydioxanone (PDO/PDS), or blend/copolymer combinations. Nonbiodegradable polymers include polyetheretherketone (PEEK), polymethyl methacrylate (PMMA), polyethylene glycol (PEG), and polyamide (PA/nylon). The choice of polymer depends on the application—for scaffolds, it is typically desirable for the material to be biodegradable so that the tissue being grown on the scaffolds fully replaces the scaffold material in the longer term. Mechanical properties, degradation behavior, processability, and biocompatibility are important factors that affect the decision of which polymer is appropriate.

With regards to additive manufacturing, thermoplastic polymers are most commonly used. They melt upon heating, which means they can either be extruded in molten form before solidification (material extrusion additive manufacturing processes—Section 11.2) or powder particles can me melted together (powder additive manufacturing processes—Section 11.2). Photocurable resins, which turn from liquid to solid form upon exposure to light in photopolymerization-based additive manufacturing processes (Section 11.2), are also used, but the cytotoxicity of resins must be considered [59,60].

For material extrusion additive manufacturing, PCL and PLA are frequently utilized. PCL is more flexible than many other biodegradable polymers and is therefore useful for soft tissue applications. Importantly, it has a lower melting point than most polymers (approximately 60°C [61] versus 159–226°C for PLA and PGA [62]), which is advantageous when printing in the proximity of living cells. Dedicated review articles for additive manufacturing of polymer melts consider the strengths and weaknesses of different polymers in more detail and discuss existing studies in the literature that have directly investigated aspects such as processability, biocompatibility and regulatory considerations [21,22,36,37].

11.3.3 Ceramics and glasses

As discussed above for polymers, there are also biodegradable or non-biodegradable variants of ceramics and glasses. A key advantage of ceramics/glasses is that they can be bioactive and therefore bond or interact with living cells. Some are made of similar constituent elements to natural bone and may release ions which stimulate new bone formation and therefore promote bone ingrowth (where bone growths through the scaffold).

Ceramics and glasses have been widely used in the clinic, but some drawbacks are their inherent brittleness and difficulty to process into complex 3D forms. Therefore, there have been significant research efforts to form composites with metals and polymers [18]. Common ceramics and glasses are hydroxyapatite (HA), dicalcium- and tricalcium-phosphate (DCP/TCP), and silicate or phosphate glasses [18,36]. The different types vary greatly in terms of their mechanical properties, degradation/corrosion rates, mechanical properties, bioactivity, and processability.

11.3.4 Metals

Metals generally have excellent mechanical properties for load-bearing biomedical applications such as bone fixation and joint replacement; particularly due their high strength, elastic modulus, and toughness. Some metals also have excellent biocompatibility (e.g., nontoxic and noninflammatory). Common metals include stainless steel, titanium, zirconium, and cobalt–chromium [18]. A major drawback is the lack of biodegradability, which limits their potential use for scaffold applications. Magnesium is biodegradable but it degrades (often referred to as corrosion) at a too rapid rate for many applications; this can be improved by alloying or coating [20,63].

11.3.5 Composites and multimaterial

All materials have their advantages and disadvantages and no single material can be truly optimal for any biomedical application due to the complex and often interrelated requirements; the material with optimal mechanical properties may not have optimal biocompatibility or manufacturing processability. Therefore, there is an extensive and growing amount research dedicated to composite materials for scaffolds and other biomedical applications [18,19]. This can be achieved through the development of new bulk materials (e.g., particle/fiber reinforced materials, polymer blends, metal alloys, etc.) or through advanced manufacturing processes such as bioprinters with multiple print heads that can fabricate structures combining multiple materials (Section 11.2).

11.4 Mechanical performance of scaffolds

As discussed in the previous section, the choice of material is potentially the most important factor affecting scaffolds mechanical performance, but in most cases, material choice is at least partially constrained by other requirements such as

biodegradation, biocompatibility, and processability. For a given material, porosity of the scaffold can be varied to influence mechanical properties, but as with material choice, porosity is often limited by other requirements such as optimization for cell viability or tissue ingrowth. However, even with a given material and given porosity, the design of the structural geometry of a scaffold can be tailored to affect mechanical performance. But again, as with material choice and porosity, the structural design may need to be satisfy other requirements such as pore interconnectivity and manufacturability. This section will summarize the effects of material choice, porosity and geometric design on mechanical performance. More detailed analysis can be found in review articles on specific topics—for example, calcium phosphate scaffolds [64], composite scaffolds [18], or polymer extrusion additive manufacturing [8].

11.4.1 Effect of material choice on mechanical performance

The elastic modulus and strength are important considerations for mechanical performance. The elastic modulus indicates the level of stress (force divided by cross-sectional area) required to deform the material and strength indicates the maximum stress that can be sustained before the material fails. The elastic modulus of scaffolds can vary by several orders of magnitude depending on the material choice [8]:

- Hydrogel scaffolds may have elastic moduli lower than 1 MPa.
- Polymeric scaffolds may have elastic moduli in the range of 3–1200 MPa:
  - 3–12 MPa for polybutylene terephthalate (PBT) [65]
  - 10–160 MPa for PCL [9,25]
  - 200–1200 MPa for more rigid polymers such as PLA [15]
- Ceramic scaffolds may have elastic moduli in the range of 150–2900 MPa [66–68].

For comparison, the elastic moduli of biological tissue are in the range of <1 MPa for soft tissues, 0.3–20 MPa for cartilage [69,70], 100–500 MPa for cancellous bone [71], and 12,000–20,000 MPa for cortical bone [71,72].

The strength of scaffolds can also vary by several orders of magnitude depending on the material choice [8]:

- Hydrogel strengths are typically lower than 1 MPa.
- PCL polymeric scaffolds may have strengths of 1–9 MPa [9,73–75].
- Ceramic–polymer composite scaffolds may have strengths of 60–130 MPa [76].
- Ceramic scaffolds may have strengths of 16–180 MPa [66,67,77].

As with elastic modulus, the range of strengths almost encompasses the range for biological tissues which may have typical strengths of 14–59 MPa (cartilage [70]), 1–12 MPa (cancellous bone [71]), or 50–190 MPa (cortical bone [71]).

Although elastic modulus and strength are commonly considered when choosing an appropriate scaffold material, it should be noted that there are unlimited different measures of material performance. Strain-at-break is often considered, to give an indication of ductility. There are many other properties and methods available to characterize mechanical performance, including directionally dependent properties, as discussed in more detail in Section 11.4.4.

11.4.2 Effect of porosity on mechanical performance

Porosity is a critically important factor that affects mechanical properties. It has been shown in many studies that mechanical performance improves as porosity is reduced [9–15]. For example, Zein et al. [14] showed an order of magnitude difference in compressive modulus and compressive strength for PCL scaffolds with porosities ranging from 48% to 77%, as can be seen in Fig. 11.4. Similar findings have been shown for other materials, including PLA [15] and acrylonitrile–butadiene–styrene (ABS) [13]. Efforts have been made to find mathematical relationship relationships between porosity and mechanical properties in tissue engineering scaffolds [13,14], and more recently predictive models have been developed to simulate porosity and mechanical properties [78].

11.4.3 Effect of scaffold design on mechanical performance

There are several aspects of geometric design that affect scaffold mechanical performance. For material extrusion additive manufacturing, the orientation of extruded filaments has been widely studied and shown to be an important factor for mechanical performance [65,73,74,79]. A typical scaffold is fabricated by laying down a lattice structure of filaments, each of which are oriented at 90° to each other on alternating layers (Fig. 11.5A–D). However, it is also possible to orient filaments at 60° to each other on alternating layers (Fig. 11.5E–H) or at other angles including 45° and 72°. A recent review suggested that 45° and 72° scaffolds suffered from reduced mechanical integrity versus 90° and 60° scaffolds due to misalignment of filaments [8]. It has

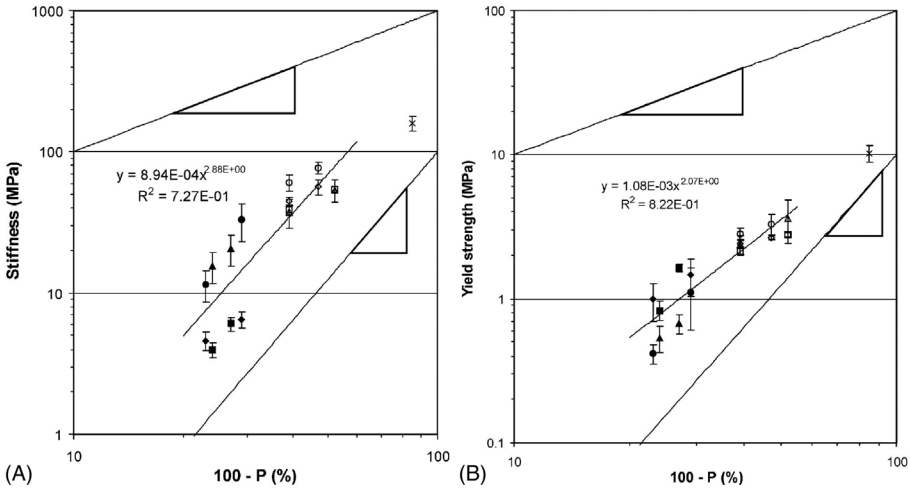

Figure 11.4 Compression modulus (A) and compression strength (B) for PCL scaffolds in the study of Zein et al. [14]. P = porosity.
Source: Reproduced with permission from [14].

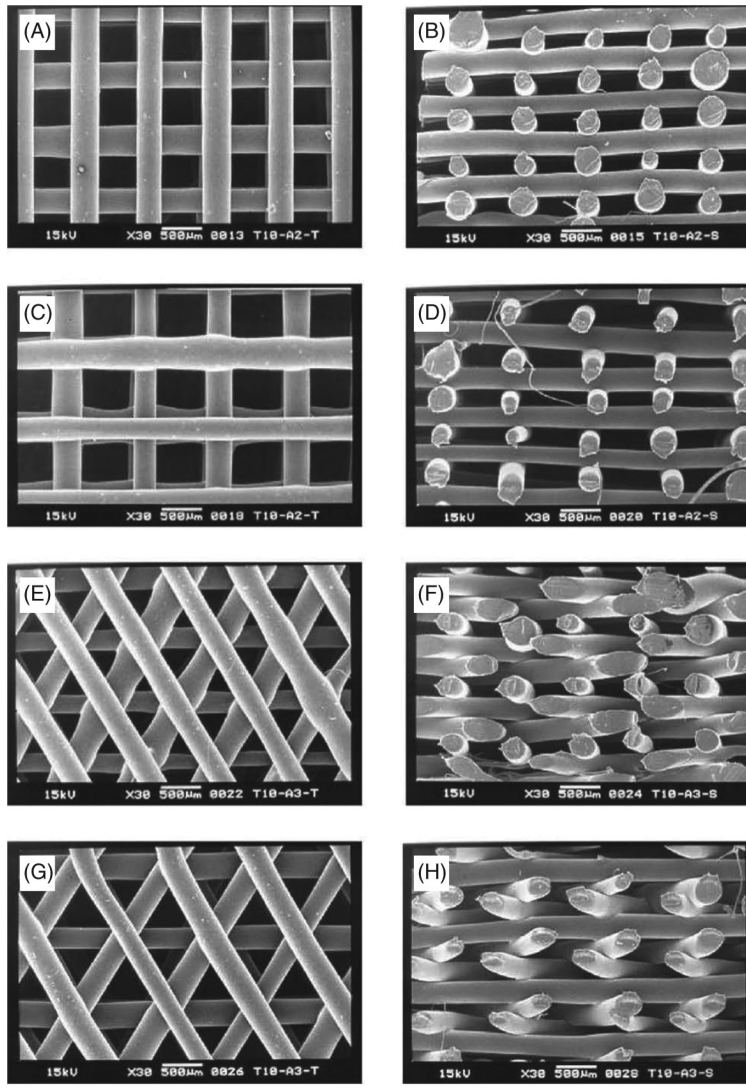

Figure 11.5 Scaffolds fabricated with material extrusion additive manufacturing with filaments oriented at 90° to each other on alternating layer (A–D) or 60° (E–H). *Source*: Reproduced with permission from [14].

been shown that the alignment of filaments across multiple layers of an additively manufactured scaffold increases the elastic modulus [80–83], which is to be expected from computer simulations as shown in Fig. 11.6. There are many other factors related to the scaffold design that affect mechanical properties, as identified in more detail elsewhere [8,84].

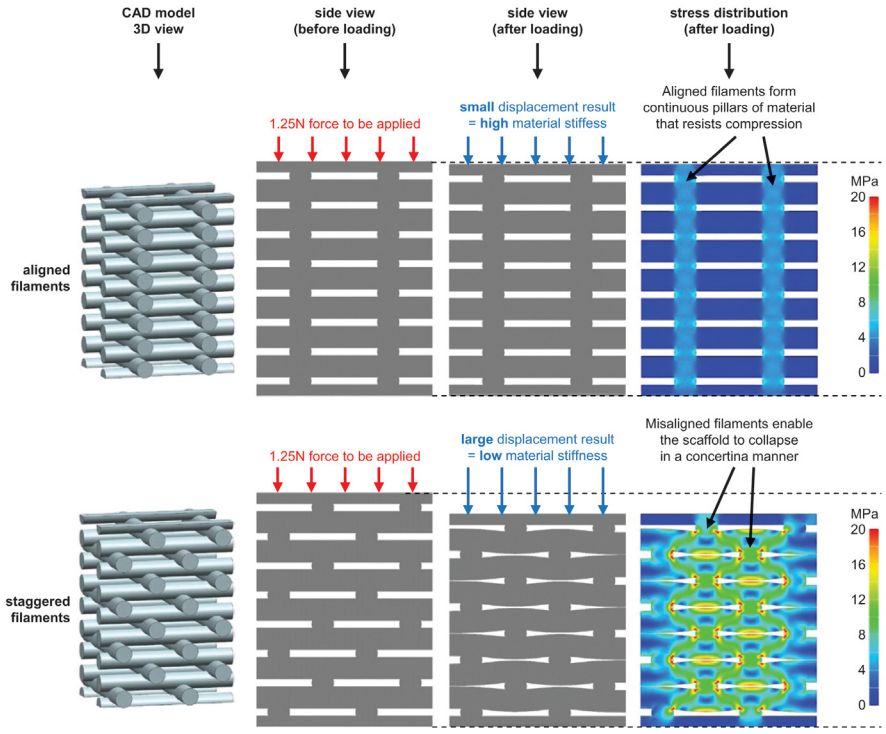

Figure 11.6 Finite element analysis simulations demonstrate how staggered filaments in a scaffold lead to a lower elastic modulus.
Source: Reproduced with permission from [8].

11.4.4 Anisotropy and other considerations related to mechanical performance

Most biological tissues and scaffold structures are anisotropic, meaning the mechanical properties vary in different directions; consider wood as an example: it can easily be split along the grain of the wood but not against the grain. Furthermore, materials may behave differently under compression versus tension. Therefore, it is critically important to consider anisotropic material properties, which have, to some extent, been neglected in much of the research to date (due to the greatly increased complexity of testing materials in multiple directions versus a single direction). In the majority of tissue engineering scaffold research, compressive properties are considered because compression tests are much simpler to perform than tensile tests—in many cases tensile tests may be unfeasible due to the relatively complex geometric requirements of tensile test specimens. Although compression tests can give useful data, many materials fail under much lower tensile loads than compressive loads. This should be considered when interpreting the data in the literature—especially for ceramics. Flexural tests are a good compromise because there are relatively simple to perform and consider a mixture of compressive and tensile loading. There are many

other considerations when analyzing the mechanical performance of materials, and numerous testing methods including shear, creep, fatigue, fracture toughness, and impact, which have been reviewed elsewhere [85].

11.5 Scaffold seeding and cell proliferation

The source of cells and the method of cell seeding (introducing cells to a scaffold) are important factors for culture of cells and tissues. They are considered in this section.

11.5.1 Cell types and cell sources

A key point for successful regeneration is the source from which the cells are harvested. Human embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), progenitor cells from a variety of tissues, and, more recently, the induced pluripotent stem cells (iPS cells) are all cell types that have been widely used [86] (Fig. 11.7).

ESCs are pluripotent in their nature and can give rise to more than 200 types of cells. The pluripotency fate of ESCs is governed by functional dynamics of transcription factors OCT4, SOX2, NANOG, and so forth, which are termed as pluripotency factors. However, the use of ECSs comes with several biologic and regulatory challenges. One of them is that the proliferative and pluripotent nature of embryonic stem cells have been associated with the development of teratomas, an obvious problem when designing clinical therapies [87].

Among the different types of stromal cells in the body, mesenchymal stromal cells (MSCs) derived from the bone marrow have been the ones most studied for clinical use. They are reasonably easy to isolate and proliferate readily, allowing for the potential to acquire them from the donors and store them in cell banks. Bone marrow-derived mesenchymal stromal cells MSCs are plastic adherent, colony-forming cells

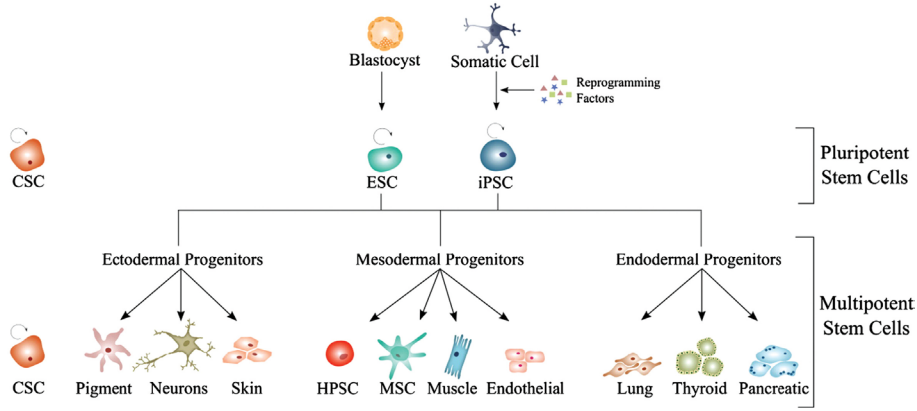

Figure 11.7 Cells types used for tissue engineering therapies.
Source: Reproduced with permission from [94].

that demonstrate the ability to differentiate into osteoblasts, adipocytes, and chondrocytes [88].

Progenitor cells (PGCs) maintain tissue homeostasis through continuous cell division, but, unlike ESCs, PGCs retain stem cells' plasticity and differentiation in tissue specific manner, giving rise to few types of cells. The ratio of PGCs population to the total cells' population may be too low, in which case their harvesting as well as in vitro manipulation is challenging and it is difficult to obtain the appropriate numbers required for therapy [89].

IPS cells are derived from somatic cells such as fibroblasts by the transfection of selected genes (Sox2, Oct3/4, Klf4, and c-Myc). Technological advancement has enabled generation of iPS cells from various kinds of adult cells phasing through ESCs or direct transdifferentiation [89]. An example of this is the work by Takasato et al. [90] where terminally differentiated skin cells were directly transformed into kidney organoids, which are functionally and structurally similar to kidney tissue in vivo.

11.5.2 Cell seeding methods

In addition to cell source, seeding of the cells onto the scaffolds is a determinant step in the attainment of the functional properties of the TE tissues. Optimization of cell seeding is essential for the successful in vitro cultivation of large tissue constructs. The key elements for cell seeding are: high yield, high kinetic rate in order to minimize the time in suspension culture for anchorage-dependent and shear-sensitive cells, and finally, spatially uniform distribution of attached cells [91].

To date, the most common method used for cell seeding is the static method, in which a concentrated cell suspension is passively introduced on a scaffold [92]. After application of the cell suspension to the graft, the construct is incubated for several minutes to allow for cell attachment. Statically seeded cells are incubated with the scaffold for several hours to several days with the goal of maximizing seeding efficiency. This technique has several limitations such as difficulty in achieving seeding uniformity, low seeding efficiency, and minimal cell penetration of scaffold walls [93].

An alternate seeding technique is the dynamic method. This method can involve rotational systems, vacuum and perfusion bioreactors. The rotational method is a system where the scaffold is rotated in a cell medium suspension at speeds of 0.2–2500 rpm for periods from 12 to 72 h [95]. The perfusion bioreactor system, as shown in Fig. 11.8, is a technique that can mimic physiological conditions such as fluid shear stress and hydrostatic pressure. It has been shown that perfusion seeding results in a high seeding efficiency (70%–90 %) with cell densities in magnitudes of 10^5–10^7 cells/cm^3 [96]. The perfusion system is advantageous for attaining even cells distribution throughout the scaffold. However, the main problem of the cell seeding in perfusion bioreactor systems is the selection of the right fluid flow velocity. Whereas high velocities do not give to the cells enough time to adhere to the material, low velocities do not move the cells fast enough, so they adhere to the tubing of the perfusion system or sink to the lowest point of the system due to gravity [97].

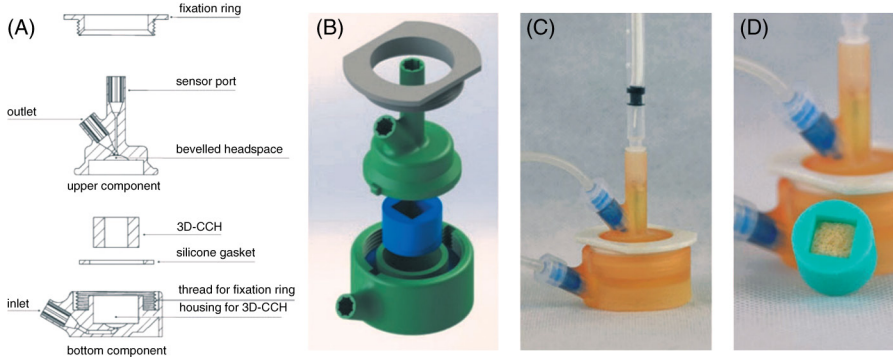

Figure 11.8 3D-printed microbioreactor for 3D cell culture: (A) section view, (B) explosion view, (C) assembled with inlet/outlet/sensor, and (D) cubic bovine bone matrix scaffold in the 3D cell culture housing (3D-CCH).
Source: Reproduced with permission from [98].

11.6 Scaffold long-term performance and tissue maturation

After seeding, scaffolds can be either matured in vitro or in vivo. The basic requirements for in vitro maturation of tissues are control of the dissolved O_2 and CO_2, pH, temperature, and nutrient concentrations. In order to aid the production of functional tissues and have a better control of the abovementioned parameters, cell-seeded scaffolds have been placed in a range of different bioreactors including orbital shakers, spinner flasks, rotating wall vessels, perfusion bioreactors, and microfluidic devices [99]. On the other hand, tissues can also be matured in vivo, as shown in Fig. 11.9. An example of this is the method for cartilage repair called matrix induced autologous chondrocytes implantation (MACI) [100,101]. In this method, autologous chondrocytes are seeded onto a scaffold, cultured for 3 days in vitro and then immediately transplanted in vivo [102]. Another example is the product Dermagraft, which is an allogeneic dermal substitute created by the combination of living neonatal foreskin fibroblasts cells and biodegradable scaffold from polyglycolic acid biomaterials [103]. The fibroblasts are immediately added to the scaffold after recovery from cryopreservation and implanted into the wound. The scaffold mesh degrades after 3–4 weeks [104].

Despite significant advances in the field, very few of these tissue engineering therapies are available for clinical use. Holoclar is one of these few therapies. Holoclar is a fibrin disc seeded with autologous limbal stem cells that aid repair of the cornea after eye burns [105]. This therapy was approved in 2015. At the time of writing, there are no tissue engineered scaffolds fabricated using additive manufacturing technologies in the market. In spite of the distinct advantages of additive manufacturing approaches, these techniques have to overcome major obstacles in order to yield successful patient-specific defect therapies. Considerations of external geometry, soft tissue

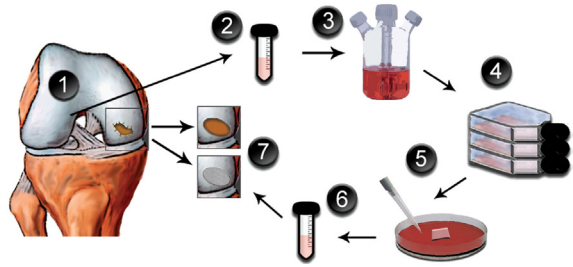

Figure 11.9 The MACI procedure. (1) Initial arthroscopy with evaluation of the injured cartilage and harvest of a full-thickness cartilage biopsy; (2) the biopsy is sent in a sterile and cooled container to the cell culture laboratory; (3) the cartilage is enzymatically digested; (4) expansion of the chondrocytes in monolayer culture for approximately four weeks; (5) the cells are seeded onto the scaffold a few days before implantation; (6) the engineered implant is sent back to the surgeon in a sterile container; (7) definitive surgery with debridement of the injured cartilage followed by implantation of the MACI-implant, which is trimmed to fit the defect size and glued with a thin layer of fibrin glue.
Source: Reproduced with permission from [107].

integration with the internal pore architectures needed to promote tissue infusion, and vascularization are big challenges. As such, optimization of these parameters in additive manufacturing approaches must occur for the translation of 3D constructs of a clinically useful size [106].

11.7 Conclusions

A wide range of manufacturing processes and materials can be used to produce tissue engineering scaffolds. Additive manufacturing is an attractive method of fabrication, and different technologies for additive manufacturing were briefly reviewed in this chapter. An overview of the materials available for scaffolds was also given. The strength and stiffness of different materials varies by several orders of magnitude. Other important considerations for mechanical properties include scaffold porosity and microstructural design. Also, as clinical usage grows, it will increasingly important to undertake anisotropic characterization of tissue engineering scaffolds in future research, to determine mechanical performance under complex multidirectional loading scenarios. In addition to mechanical requirements, other important considerations for scaffold materials and geometric design are processability, biocompatibility/bioactivity and biodegradability. Some scaffolds can be implanted without any biological component, such as those designed for bone ingrowth. However, for many tissues, and for organs in the longer term, scaffolds must be seeded with cells prior to transplantation. This chapter reviewed several alternative cell sources and seeding methods and gave an outlook on tissue maturation.

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3D printing equipment in medicine

12

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Chapter outline

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12.1 Introduction

Three Dimensional (3D) printing also termed as additive manufacturing (AA) or Rapid prototyping (RP) or solid free-form technology (SFF) is a manufacturing technology which fabricates the objects or devices by fusing or depositing the materials such as plastics, ceramics, metals, powders, liquids. or even living cells in layers to generate a three dimensional object [1].

12.2 Evolution and history of 3D printing

Journey of 3D printing dates back to 1980 with attempts of Dr. Kodama who described a a layer-by-layer approach for the fabrication of materials which eventually led to the development of Steriolithography (SLA). He created a photosensitive resin polymerized by UV light. In 1986, Charles Hull filed the first patent for Steriolithography and founded 3D system corporation and launched SLA-1 in 1987. In 1988, Carl Deckard from University of Texas brought a patent on one more additive manufacturing method, that is, Selective Laser Sintering (SLS) technology. He used this technology to fuse the powdered grains together using laser. Around the same time Scott Crump filed a patent on Fused Deposition Modelling (FDM) or Fused Filament Fabrication (FFF) which developed as a third main 3D printing technology (https://www.sculpteo.com/blog/2016/12/14/the-history-of-3d-printing-3dprinting-technologies-from-the-80s-to-today/). From 1993 to 1999 3D printing sector showed a substantial development and different types of printers and tools were developed for professional and individual usage. It was during this time that different types of CAD tools were developed for 3D printing which added up the versatility to the area (https://www.sculpteo.com/blog/2016/12/14/the-history-of-3d-printing-3dprinting-technologies-from-the-80s-to-today/). With the advancements of 3D in printing, healthcare industry embraced this technology with the hope of adding up the precision to the surgeries and making the procedures simple. Pioneer to the adoption of 3D printing in healthcare came up with the series of trails conducted by team of scientists at Boston Children's Hospital. Team fabricated scaffolds from collagen and other synthetic polymers for the replacement of urinary bladders. Synthesized scaffolds were seeded with cells from patients and were allowed to mature into a functional organ. Initial trials with small group of patients were successful but the process of fabricating the scaffolds by hand was very labors and had reproducibility issues associated. Therefore, the next logical step to upgrade this process was to automate the whole procedure [2]. Process was initiated by Dr. Atala who moved to Wake Forest Baptist Medical Centre and his efforts led to the formation of Wake Forest Institute for Regenerative Medicine (WFIRM). Researchers at WFIRM began conducting experiments with basic inkjet desktop printer. Upgradation of this basic printer led to the development of a machine which was capable of printing customized scaffolds for other human organs. In 2006 Centre for Applied Reconstruction Technology in Surgery (CARTIS) was formed and since then they have made a rapid progress. They

now offer highly developed surgical 3D printing services such as custom implants and prosthesis [2]. CARTIS made a breakthrough research in 2013 when they reconstituted victims face crushed in a motorbike accident. Team of surgeons performed multiple surgeries on victims face and reconstituted it by specially designed titanium plates fabricated using 3D printing technology (https://www.mirror.co.uk/news/technology-science/science/mans-crushed-face-rebuiltafter-2804056) [3]. Furthermore, other pivotal research work during 2012 and 2014 in 3D printing area made the patient life easier which further boosted the area. In 2012 using cutting edge laser manufacturing technology doctors were able to fabricate layers of titanium to form custom metal jaw bones to replace the infected jaw of a 83-year old female (http://www.nhs.uk/news/2012/02February/Pages/3d-printing-jawbone-implantcreated.aspx). In 2014, an orthopedic surgeon at Newcastle Upon Tyne Hospital NHS trust treated a patient in his 60s who had lost half of his pelvis to bone cancer. Dr. Craig created a new pelvis using 3D printing technology using titanium powder (http://www.telegraph.co.uk/health/10627556/Surgeon-creates-pelvis-using-3D-printer.html). In 2014, ground breaking hip and stem cell surgery in Southampton General Hospital proved to be a game changer in the history of 3D printing. 3D printed hip was made from titanium with additional features. Doctors inserted a graft between implant and pelvis containing stem cells which acted as a filler for loss of bone. Graft was implanted with patients own bone marrow cells. This revolutionary procedure provided numerous benefits to the patient. In addition to use of titanium which made the hip more durable additional bone graft material was used to induce the regeneration of the defect. So this procedure introduced the concept of "reconstruction and regeneration" in the area of 3D printing (http://www.bbc.co.uk/news/uk-england-hampshire-27436039). Another major advance in 3D printing technology was accomplished by the replacement of most of a human skull with 3D printed plastic one which saved the life of a patient. Surgeons at University Medical Centre Utrecht operated a 22-year old female who suffered from chronic bone disorder. Patients top section of the skull was removed and was replaced with a 3D printed implant. This surgery was a milestone in the area of 3D printing as it was the first instance of a successful 3D printed cranium (http://io9.com/take-a-look-at-the-first-successfully-transplanted-3d-1553869043). In addition to the contribution of 3D printing technology to the orthopedic procedures other areas are also witnessing a substantial progress major being the area of 3D bioprinting which claims to replicate the human tissue and organs. In 2015, Cardiovascular Innovation Institute (CII) announced that they can build all five parts of heart (i.e., Valves, coronary vessels, microcirculation contractile cells and the organs electrical system) using a "biological architecture tool" (BAT) or "Bioassembly tool." All the parts eventually will be assembled into an bioartifical heart which can prove a relief to millions of patients with severe cardiac aliments [3]. In 2016, team of regenerative medicine scientist from Wake Forest Baptist Medical Centre printed ear, bone, and muscle structure. Scientists developed an integrated tissue - organ printer (ITOP) which is able to deposit biodegradable polymers and can pattern multiple cell laden composite hydrogels. 3D printed organs produced from this study were tested on mice and results were encouraging [4]. In 2016, scientists at Harvard created a 3D printed renal architecture which can increase the life

expectancy of patients suffering with chronic kidney failure or other kidney ailments. Scientists used a 3D printed silicon gasket as a mold onto which they deposited a layer of engineered extracellular matrix composed of gelatin/fibrin hydrogel. After printing the initial structure scientists printed fugitive ink (printed material which is ultimately liquefied and removed) is printed in a convoluted winding tubular structure followed by one more layer of ECM. Entire set up is then cooled down to remove the fugitive ink leaving behind the open tubular structures within the ECM. Whole 3D printed structure mimics the architecture of kidney. Open spaces left after the removal of fugitive ink are then seeded with living cells those attach to ECM and start proliferating. This device needs further pre-clinical trials in lab animals for the validation of its efficiency in patients [5]. In 2018, scientists at Newcastle University fabricated a 3D printed cornea which in future can ensure an unlimited supply of corneas. Scientists prepared a bio-ink using stem cells from a healthy donor mixed with biopolymers such as alginate and collagen. Formulated bio-ink was extruded in concentric circles to form a shape of human cornea. This study needs further experimentation before bioengineered corneas can be transplanted into humans [6]. Most recent advancement in the area of 3D printing came in early 2019 when scientists at WFIRM created a mobile skin bioprinting system which can address the limitations of currently available skin grafts. Researchers created a skin bioprinting system conjoined with integrated imaging technology device that scans the wounds and delivers the cells in "*layer-by-layer*" format to maintain the natural architecture of the intact skin. This study is a proof of concept and further extensive experimentation and clinical trials before it can be used to treat human wounds [7].

12.3 Process of creating 3D models or How 3D models are created

3D models for medical applications are created by *"Digital Imaging and Communications in Medicine"* which is a common database for storing and transferring medical images obtained from the patients [8]. For creating a custom prosthesis or a medical implant using 3D printer, the following steps are involved:

1. Acquisition of image data
2. Segmentation or extraction of chosen region of interest (ROI)
3. Creating a 3D mesh
4. Transformation of data to 3D printer for model generation

12.3.1 Acquisition of image data

To create an opposite biomimic, it is very important to understand the external and internal architecture of the target tissue or origin [9]. During image acquisition following, two parameters play a very important role in deciding the quality of model. First one is the selection of a proper image data as low resolution images can lead to the incongruity between actual anatomy and the model being generated [10]. Second one

being the slice thickness which is dependent on the complexity of the organ or tissue in question. For example, for creating a maxillofacial model slice thickness should be between 0.5 and 1 mm, whereas for pelvis and long bones, good models can be created suing the slice thickness up to 2 mm [11].

Most common imaging modulations used for obtaining patient specific information are as follows:

12.3.1.1 Computed tomography (CT)

This imaging tool uses X-rays (ionizing radiation) to create 2D images. 2D images are then stacked via tomographic reconstruction algorithms to generate a 3D view. CT performs better in imaging hard tissues like bone or tumor [9].

12.3.1.2 Magnetic resonance imaging (MRI)

MRI uses pulsed radio frequency electromagnetic waves. It detects excited radio frequency signal from hydrogen atoms on the sample creating an image based on the time taken for protons to realign with magnetic field as well as amount of energy released. Individual 3D images are stacked together to create an MRI image. This technique is used in imaging the soft tissue [12].

12.3.1.3 Ultrasound imaging

This technology uses sound energy to scan the patient. Ultrasound waves are produced by the transducer which can both emit and detect the sound waves to generate computer images. Using ultrasound elastography mechanical properties of the tissue can be measured quantitatively which provides a good help to build a model with same mechanical profile to the original tissue [13].

12.3.2 Segmentation or mesh creation

This is an optional step in the generation of 3D printed models but considering the practical scenario this step is frequently used in medical applications. Segmentation is a post processing step which is used to generate the area of inters within the given data set. It extracts a surface from segmented data to generate a surface mesh [14]. Segmentation relies on various tools for the generation of an ideal image. First one being the “threshold tool” which allows to set a range of values from the data to be retained while ignoring the data that falls out of the range. This tool is useful in retaining or removing the area of interest based on the density of tissue type. For example, this technique can differentiate bone from the remaining soft tissue due to differences in density [15]. Another technique is “seed-based region growing” here operator selects areas from the image using the start point or a seed and sets a voxel density parameter. Additional voxels are subsequently added those meet the defined density criteria. Data from the voxels are then converted into a mesh composed of series of triangular facets. Any artifacts can be smoothened out using manual or automated algorithms [16].

12.3.3 3D modelling

Final step in the generation of an ideal 3D tissue model is designing the internal architectures namely channels, pores, etc. those can enable cell attachment, proliferation, nutrient flow, tissue maturation, etc [17]. 3D model generated from image segmentation is usually represented as STL file format which is the most compatible for majority of the 3D bioprinters [18]. Main approaches used in designing the internal architecture of the tissue construct are as follows: [9] (15):

1. CAD-based design
2. Image-based design
3. Freeform design
4. Implicit Design
5. Space filling curves.

CAD-based design system employs different approaches to design the tissue architecture, for example "Constructive solid geometry (CSG)" generated design models based on solid primitives and Boolean operations [19] "B Rep" uses boundary elements to define the geometry, and Spatial Occupancy Enumeration (SOE) represents solid objects using cubic unit elements [9]. Fig. 12.1 gives the details of computer-aided design-based systems constructed from different primitives. Different research groups have used and modified above mentioned techniques for the generation of desired tissue model. Chean et al. designed 3D bone construct with controlled porosity by designing algorithms enabling subtraction of negative geometry using a CAD model [20]. To generate a tissue model with irregular pores Hollister et al. used image-based approaches. They designed a patient specific craniofacial biomaterial scaffold from CT and MRI data, and a model was generated by filling up the defected region with the binary unit cell [21]. Furthermore, other research groups worked on designing tissue models with controlled architecture together with desired material composition. Smith et al. used image-based design and computer software to generate a precisely sized and shaped scaffold for osseous tissue regeneration. Model was generated by employing selective laser sintering, ploycarbonate was used to create a condylar ramus unit (CRU) scaffold for the reconstruction of tempomandibular joint [22].

12.4 Process of 3D bioprinting

Major technologies those are used for the deposition and patterning of biological materials for the generation of 3D tissue models are listed as follows:

1. Laser-based bioprinting
2. Droplet-based bioprinting
3. Extrusion-based bioprinting
4. Sterolithography bioprinting

All the four listed technologies are further classified into different categories depending on the process, detailed schematics of bioprinting process is depicted in the

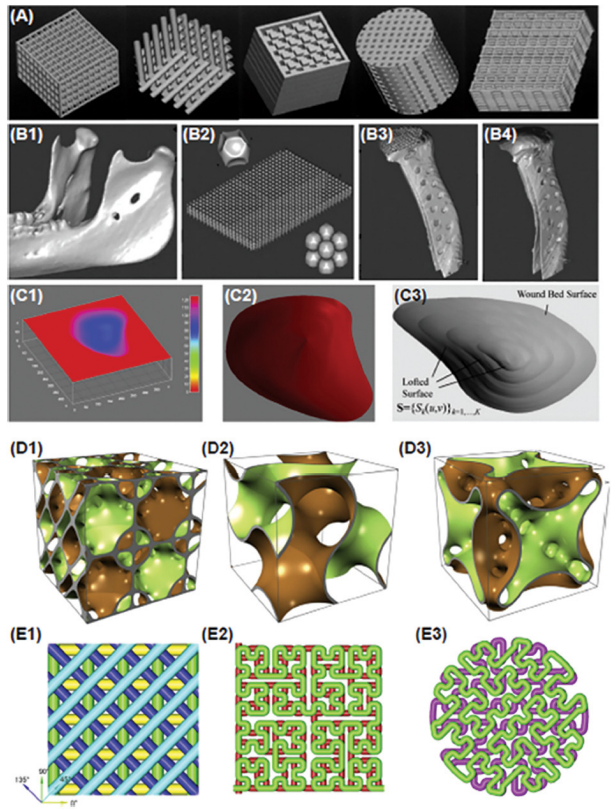

Figure 12.1 (A) Computer-aided design-based systems constructed from different primitives. (B1)–(B4) Image-based design of mandibular condylescaffolds. (C1)–(C3) Freeform design of a wound device. (D1)–(D3) Triply periodic minimal implicit surfaces. (E1)–(E3) Space-filling curves.
Source: Reproduced with permission from Vijayavenkataraman et al. [9].

flow diagram (Fig. 12.2). For any type of bioprinter, selection and optimization of *bioink* play a crucial role in the generation of a final 3D tissue model. An ideal bioink should be highly biocompatible to support living cells, mechanically stable, and should provide high resolution during printing [23]. Examples of bioink includes cell suspension, cell-laden hydrogels, microcarriers, cell/tissue spheroids, and decellularized matrix components [24,25]. Characteristics of the bioink decides its applicability, for example, biostable hydrogels like those made from PEG, alginate or agarose manifest strong mechanical properties so they are used for bioprinting of cartilage [26–28]. Bioactive hydrogels such as gelatin, collagen, or fibrin those support cell adhesion and proliferation have applicability in the area of cardiovascular and hepatic bioprinting as cardiomyocytes need a congenial environment for growth and proliferation [29,30]. Fig. 12.3 gives the detailed working of different types of bioprinting approaches.

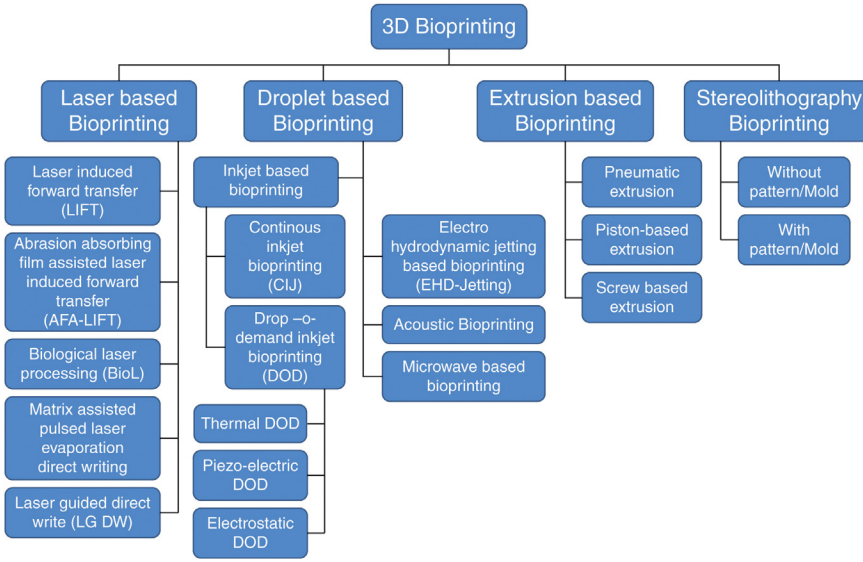

Figure 12.2 Classification of 3D printing.
Source: Reproduced with permission from Vijayavenkataraman et al. [9].

12.4.1 Laser-based bioprinting (LAB)

This is a non-contact nozzle free printing process which was originally developed for high resolution patterning of the metals such as Ag, $BaTiO_3$, and NiCr [31]. Process utilizes laser energy to pattern bioink in 3D spatial arrangement with the help of CAD/CAM tool [9]. Typical LAB device has the following components:

1. pulsed laser beam
2. a focusing system
3. "ribbon" which contains a donor transport support made from glass which is further covered by a layer of laser-energy-absorbing layer like gold or titanium
4. biological material or bioink
5. receiving sub-facing ribbon

Material directs laser pulses through ribbon containing desired solution of the bioink, for example, cells mixed with a compatible polymer. Bioink is suspended on the bottom of the ribbon which when vaporized by laser pulse creates a high-pressure bubble which lands on the receiving substrate placed beyond the ribbon in the form of a droplet. Desired 3D structures are created by repeating the process [32]. According to the type of laser source and laser transparent print ribbon process have slight variations as discussed. LIFT is commonly used for cell patterning and biopriting tissue constructs [33]. AFA-LIFT and Bio LP use a thick sacrificial layer which reduces the exposure of the cells to laser thus reducing the risk of cell damage [34,33]. MAPLE DW and LG DW uses low power laser pulses to avoid the cell damage [35].

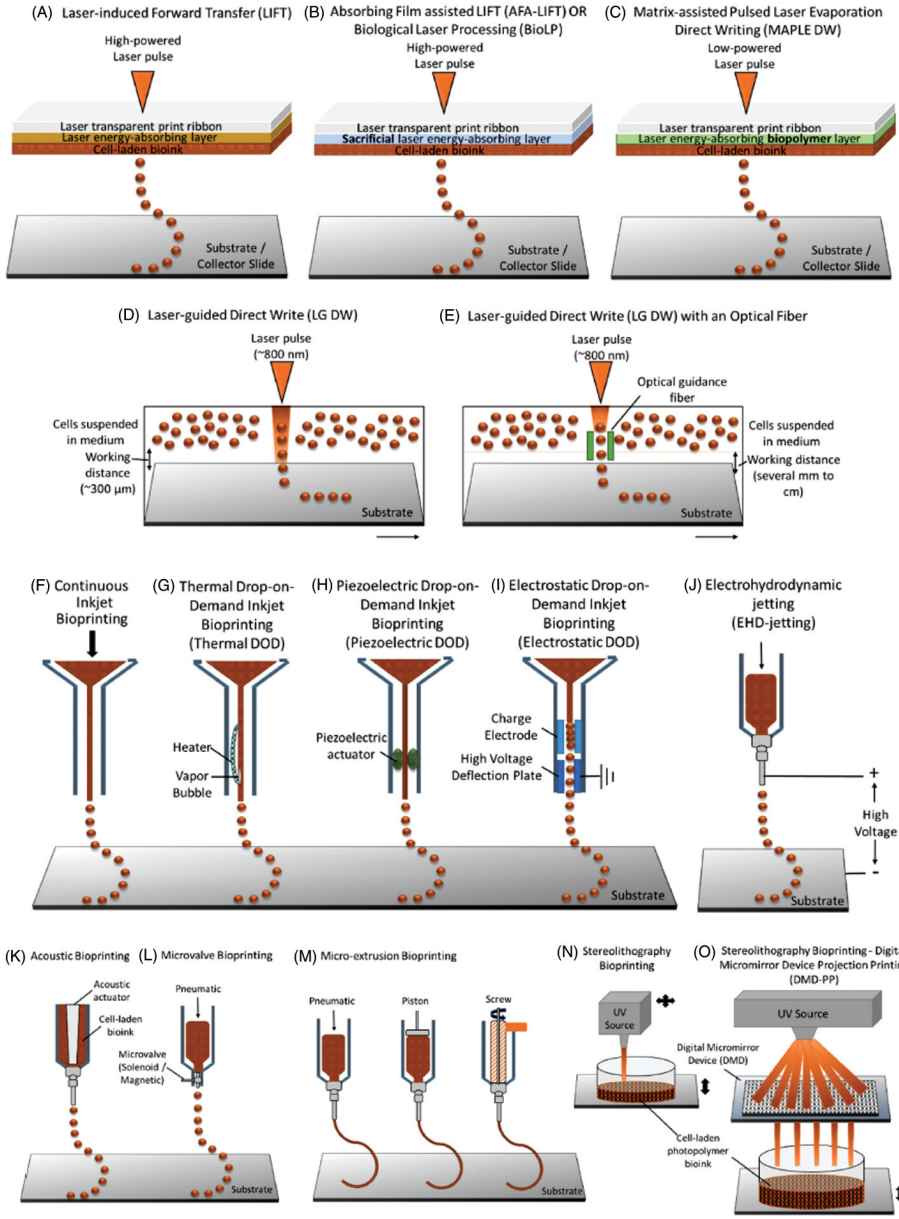

Figure 12.3 Working of different types of 3D printing equipment's.
Source: Reproduced with permission from Vijayavenkataraman et al. [9].

Applications of LAB in medicine: Michael et al. created fully cellularized skin substitute using LAB. Skin substitute was created by positioning fibroblasts and keratinocytes on top of a Matriderm which acted as a matrix. Whole process was facilitated

by a unique feature of LAB which makes it possible to position the different cell types in exact 3D spatial pattern [36]. For *in vivo* application, LAB has been used to deposit nano-hydroxyapatite in a mouse calvaria defect model. Future research is focused on the development of materials those can integrate with the patient's tissue. Advances might also open the avenues to incorporate patients own cells which will facilitate the applicability of these constructs [37].

12.4.2 Droplet-based bioprinting (DBB)

This process ejects the droplets of bioink on a predefined location onto the desired substrate [9]. DBB is further divided into inkjet, acoustic-droplet ejection, and microwave bioprinting (Fig. 12.2). Inkjet-based printing is further divided into CIJ and DOD based on the working. DOD printing uses thermal or piezoelectric actuators or electrostatic forces to generate droplets. EHD printing uses high electric voltage to eject the droplets, whereas acoustic printing uses acoustic waves to generate the droplets [37].

Applications of DBB in medicine: DBB has shown wide applicability in the area of tissue engineering and regenerative medicine. Cui et al. engineered bone like tissue with increased compressive modulus using TIJ printer [38]. Cardiac tissue with beating response was engineered using HL1 cells on alginate hydrogels. Cardiac tissue mimic was engineered by biopronting consecutive layers of calcium chloride using TIJ bioprinting [39]. Similarly, Atalas et al. fabricated cartilage tissue using microvalve bioprinting by printing chondrocytes, fibrinogen, and collagen onto defined locations over the PCL fibers [40]. Other more complex tissues such as liver, lungs and neural tissues have also been fabricated using DBB [37]. There is a single clinics case which demonstrated the transplantation of a 3D printed biodegradable airway splint fabricated from PCL into an infant. Further developments in the technology are expected which would pace up its transition to clinics.

12.4.3 Extrusion-based bioprinting (EBB)

EBB is most widely used of all bioprinting methodologies. In this type of printing bioink is extruded out of the nozzle using either pneumatic pressure or with the help of mechanical forces with the help of a piston or a screw [9]. EBB has associated advantages such as scalability which makes it feasible to print human tissue and organs using this technology. Other advantage is the capacity to print high viscosity (~600 Kpas) bioinks at high cell densities which is a prerequisite for printing any complex human tissue or organ [40]. EBB has associated limitations such as low resolution in comparison to other printing technologies and nozzle clogging [9].

Applications of EBB in medicine: Considering the capacities of EBB, it has been widely used for the engineering of complex human tissue and organs namely lungs, liver, skin, etc. Billet et al. engineered artificial live tissue construct using hepatocytes on gelatin methacrylamide hydrogels [41]. Complex tissues such as lung were engineered by Horvath et al. using EBB printing [42]. Other group bioprinted human adipose derived mesenchymal stem cells (hASC's) loaded with decellularized matrix

components [42]. All these initial developments in the area of regenerative medicine are expected to revolutionize the area of medicine and surgery and also tackle the issue to limitations of human organs for transplantation.

12.4.4 Stereolithography bioprinting

This technology involves curing (or polymerization) of a layer of photopolymer resin by light (UV). Light movement is controlled by computer code/images/CAD files forming a 3D structure. Depending on the movement of light source, there are two modalities of stereolithography. In the first one light source is computer controlled and moves as per the structure required in each layer of the 3D object. Second modality employs digital micromirror device (DMD) which constitutes of an array of several thousand micro-mirrors. Bioprinting process which involves use of DMD is called DMD-PP (digital micromirror device-Projection printing). Each of the micro-mirror could be controlled to reflect light in spatial pattern which facilitates the polymerization of a whole layer at once [9,43].

12.5 Applications of 3D printing in pharma industry

3D printing is gaining importance in the field of pharmaceutical research because of its ability to synthesize tailored-made formulations that can be applied to personalized therapy/medicine. Major focus of the development is patient centered dosage. There are two major forms of dosage delivery (1) oral dosage and (2) topical dosage. Tailoring the dosage according to patients need has proven to be cost effective and convenient for patient. Together with tailoring, the drug tempering the manufacturing process to orient it to patients needs would revolutionize the pharma industry.

12.5.1 Application of 3DP technology for oral dosage form

Tablets produced by 3DP can be categorized: (1) Single active pharmaceutical ingredient (API) tablets. (2) Multiple active pharmaceutical ingredient (API) tablets

12.5.1.1 Single active pharmaceutical ingredient (API) tablets

High drug loaded single API dosage forms are being successfully prepared by 3DP technology. For example, a thermoplastic polyurethane-based dosage form loaded with 60% drug was successfully developed via FDA method [44]. 3DP is also being explored for the manufacturing of extended release (ER) tablets. Skowyra et al. fabricated ER tablets using predinisolone loaded polyvinyl alcohol filaments, formulation was able to release the drug for up to 24 h [45]. Selection of 3DP materials and methods has a dramatic influence on the drug release profile. Wang et al. developed a pseudoephedrine hydrochloride dosage, wherein drug release rates were adjusted by varying the proportion of Kollidon and hydroxypropylmeythyl cellulose (HPMC) [46]. Novel productive technology like 3D extrusion-based printing technology has

been explored in the pharmaceutical industry. This technology has been used for the fabrication of gastro-floating tablets [47]. Floating drug delivery system has advantages over the conventional delivery system. In floating system, drug is released slowly at a desired rate from the system and residual system is emptied from the stomach. This results in the increased gastric retention time (GRT) and more controlled plasma drug concentration over time [48].

12.5.1.2 Multiple active pharmaceutical ingredient (API) tablets

"Polypills" or multiple APIs combine complex medication regimes into one. Khaled et al. produced a polypill to treat hypertension patients with diabetes. This polypill was composed of a captopril osmotic pump compartment, a joining layer and a sustained release compartment of nifedipine and glipizide [49]. 3DP is also being applied to control more complex release profiles. Using complex templates, it is possible to create tablets that contain multiple components which can generate a multi-action releasing profile or multiple pulse drug release. Sun and Son used 3DP method to fabricate customized tablets; those can achieve any desired release prolife. Core components of the tables include surface eroding polymer with drug, surface eroding polymer without drug and an impermeable polymer that forms a protective coating (Fig. 12.4). Surface eroding polymer with drug can be fabricated into varying shapes using 3DP which can lead to different drug release profile. Changing the shape of the surface eroding polymer with drug can lead to constant release, increased release, decreased release, or pulse release which can be synchronized with the patient requirements. For example, pulse release can be used for the drug that needs to be harmonized with the biological clock of the patient [50]. Polypills can help patients by reducing the number of pills that they need to take per day, and it can also improve patient compliance thus improving the healthcare [44].

12.5.2 Application of 3DP technology for topical dosage form

Topical delivery is the next preferred model of delivery after oral. Methods of delivery include implants and microneedles.

12.5.2.1 Implants for topical delivery

3DP-based implants are designed as a single device for multiple API loading which helps to achieve precise and targeted delivery of the drug. Ahlfeld et al. fabricated a bone healing scaffold with multiple API's loaded inside with the help of 3DP. In this formulation calcium phosphate cement was combined with VEGF followed by loading onto hydrogel strands [51]. Wu et al. designed a multiple drug implant for the treatment of bone tuberculosis by incorporating isoniazid and rifampcin into each layer of the implant designed in the form of concentric cylinders. Formulation was found to be effective for the treatment of bone TB. Because of their efficient cytocompatibility which can be altered by the choice of polymer used in the implant, 3DP-based multiple drug implants could be promising approach for the treatment of bone TB [52]. Patch-like implants are the recent type in implants fabricated for extended drug release

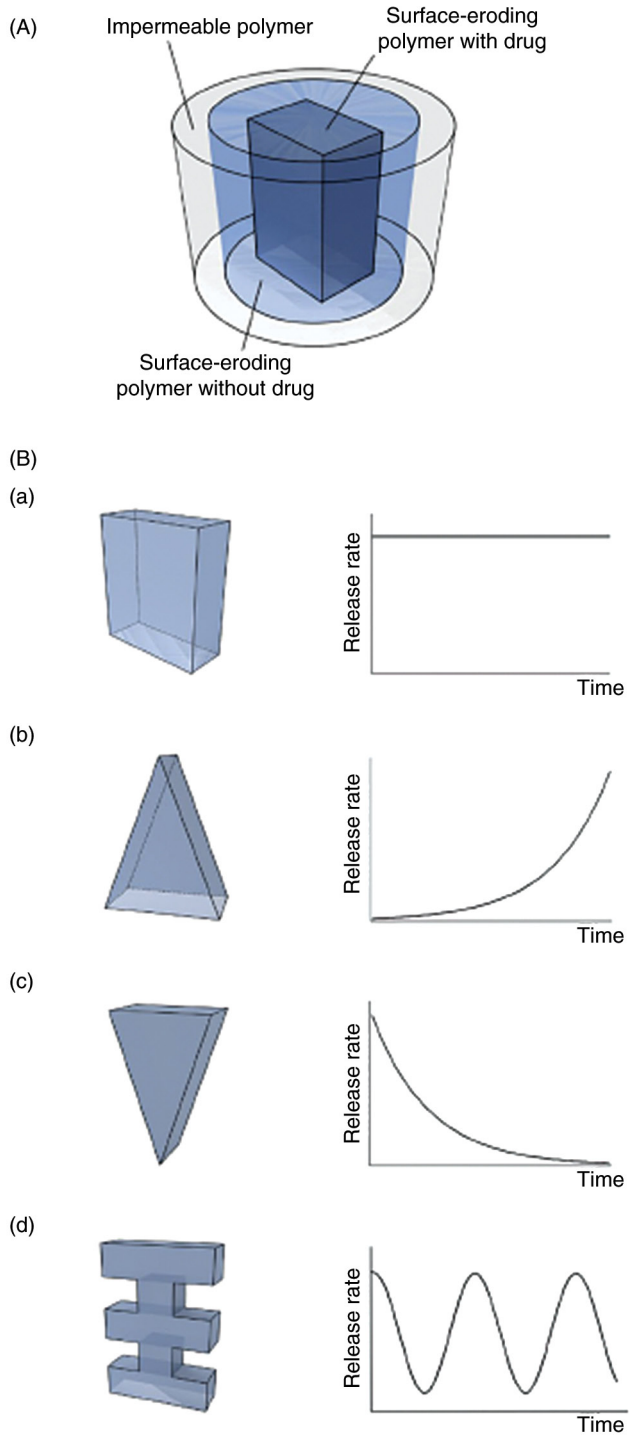

Figure 12.4 Structural examples of release-controlled tablets. (A) Scheme of the release-controlled tablet, (B) examples of drug release profiles from each surface-eroding polymer in different shapes, representing constant drug release (a), increasing drug release (b), decreasing drug release (c), and multiple-pulse drug release (d), respectively.
Source: Reproduced with permission from Park et al. [44].

for chronic diseases such as cancer. Patch made from poly-lactic-co-glycolic acid, polycaprolactone and 5-fluorouracil was directly attached to the pancreas of an athymic mice model showed significant efficiency in reducing the pancreatic cancer [53].

12.5.2.2 Microneedles (MN) for topical delivery

MN are the array of micro-sized needles on the surface of a matric which may be more effective as a delivery vehicle than a patch. Lu et al. fabricated MN for skin carcinoma using microstereolithography. System was made by mixing poly (propylene fumarate) with diethyl fumarate and achieved the controlled release of dacarbazine for 5 weeks to reduce the tumor. Pere et al. fabricated biocompatible formulation for the delivery of insulin. Developed MN released insulin rapidly within 30 min regardless the shape of needle, this system can prove very helpful for diabetic with spiked sugar levels. Patient specific care is being revolutionized by 3DP. Recently 3D scanning system allows acquiring the information regarding skin features which has helped researchers to fabricate a tailored patch which is expected to enhance patient compliance and at the same time will have enhanced efficiency [44]. Fig. 12.5 gives the details of 2D and 3D printing applications for transdermal drug delivery.

12.5.3 3D FDM-printing of personalized medicine and digital pharmacies

Pharma industry and prescription drug area are predicted to be revolutionized by 3D printing. 3D-FDM infrastructure can very well integrate with the current compounding pharmacies to make the personalized medicine a reality. 3D printing equipment's could be set over existing benches in the solid or liquid preparation rooms [54]. With

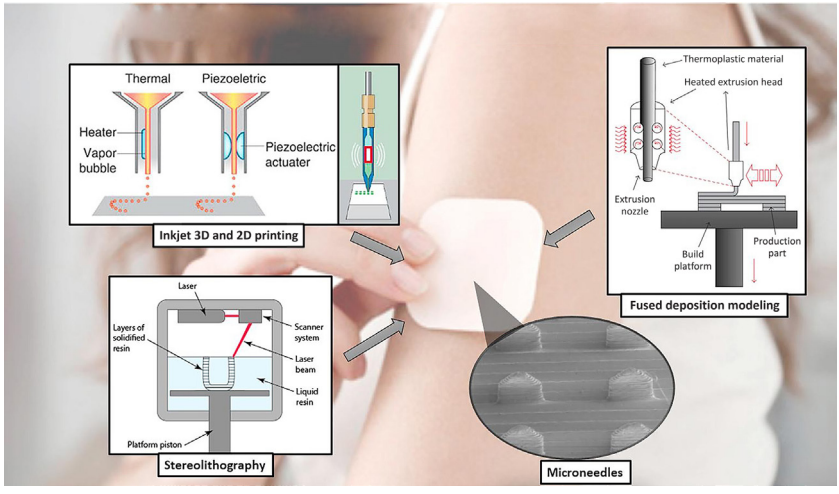

Figure 12.5 2D and 3D printing applications for transdermal drug delivery.
Source: Reproduced with permission from Economidou et al. [134].

the small investment it is possible to transform a compounding pharmacy into a digital pharmacy. In terms of operations whole process can be divided into few steps which will be handled by a trained technician. Process will allow the drug to be dispensed to the patient (with a desired dosage and shape) in the reception room [55]. Manufacturing the drug using 3D FDM will also allow to modulate the drug release from the printed medicine, and drug release kinetics can also be adjusted by adjusting the printing parameters thus personalizing the formulation according to the patients' needs [56]. Over the time, there have been cases of intoxication related to the use of medications due to the error in the weight of the active ingredient [57]. 3D FDM would lower this risk by reducing the number of steps involved in the process and plummeting the human intervention. For quality control of 3D printed formulations newer analysis tools can be used, for example, near infrared spectroscopy (which can perform batch to batch analysis) or Terahertz pulsed imagining which allows the acquisition of single depth scans in few milliseconds. These innovative analytical approaches can cover the safety related issues of the 3D FDM making it more safe and robust for patients [57,58].

12.6 Applications of 3D printing in medical education

As 3D printing is evolving it is being explored in different areas to add the precision and accuracy. 3D printing has shown application the area of general medical education, surgical education as well as in patient education. Following section gives the details about applications of 3D printing in medical education.

12.6.1 General medical education

3D printed models can be used effectively as a teaching and learning aid to get a better understating of anatomy of different human organs and associated pathologies. Human anatomy is conventionally taught through the use of human cadaver which will soon be replaced by the 3D printed models mimicking human anatomy [59]. In randomized control trails by Li et al. 120 medical students were examined on their understanding of complex spinal anatomy. Examination was done through teaching modules using CT image, 3D image or 3D printed models. Results indicated that 3D printing model group showed superior results over the control groups [60]. Additional research studies proved that 3D printed models were superior to book or digital learning. Furthermore, 3D models gave a better understanding of organ function, pathologies, and disease progression [61].

12.6.2 Simulation training

Simulation training on 3D models assists trainees to explore the organ anatomy and at the same time help them develop the better understanding of the organ in question. Trainees/medical students working on the 3D models found that these models were

helpful in enhancing their surgical skill sets [10]. Working with 3D models also created a comfortable environment for trainees to learn from their own mistakes [62,63]. In a study done by Mashiko et al., students were trained to clip an aneurysm through use of a 3D haptic model. Exposure of students to the simulation models helped them to get the better understanding of the critical things such as "clipping direction", "election of clip", or "shape of aneurysm" which gave them a better understanding of the procedure prior to the actual surgery. Survey results reported excellent (83%) to good (16%) in regard to how 3D printed models increased the knowledge about the patient aneurysm.

12.6.3 Surgical education

Simulation can be a very vital tool for planning and execution of critical surgeries. Using the patient specific 3D models staff and surgeons can appreciate patient specific anatomy and can also map out the best surgical routes which can make the procedure more efficient [59]. Gerstle et al. suggested that use of 3D models in surgeries helped surgeons to handle possible complications and also reduce the operating room times [61]. Furthermore, an analysis done on the residents those were using 3D models indicated that 60% of the participants reported them as "very much useful" and 40% as "very useful" [64]. 3D models have also been used for intraoperative guidance tool. For example, 3D modeling may be helpful to the surgeons to orient themselves while operating which might be an important parameter for complicated anatomical sites [65]. 3D models have also been used for postoperative evaluation of the patient. Torres et al. reported that physicians were able to use the patient model to analyze the accuracy of an orthognathic surgery post-surgery [66]. Fig. 12.6 represents the schematics of the steps involved in preoperative planning of surgery [67].

12.6.4 Patient education

Use of pre- or post-operative 3D surgical models for patient education has augmented patient understanding of the procedure and possible outcomes. In a study conducted on patients and their families 10 participants rated these models to be "very high value" and remaining 2 rated them as "high value" [64]. In other study, where 3D patient model was created to provide a preoperative guidance to the patient. Patient responded max positive value (5/5) in providing the information about the upcoming procedure and also helped the patient to understanding the possible outcome of the procedure [68].

12.7 Applications of 3D printing for generation of *in vitro* disease models

Conventionally animal models such as transgenic mice with specific gene alterations represented the best fit to mimic any disease. But the major disadvantage with these models is the discrepancy in the molecular mechanism with the same disease

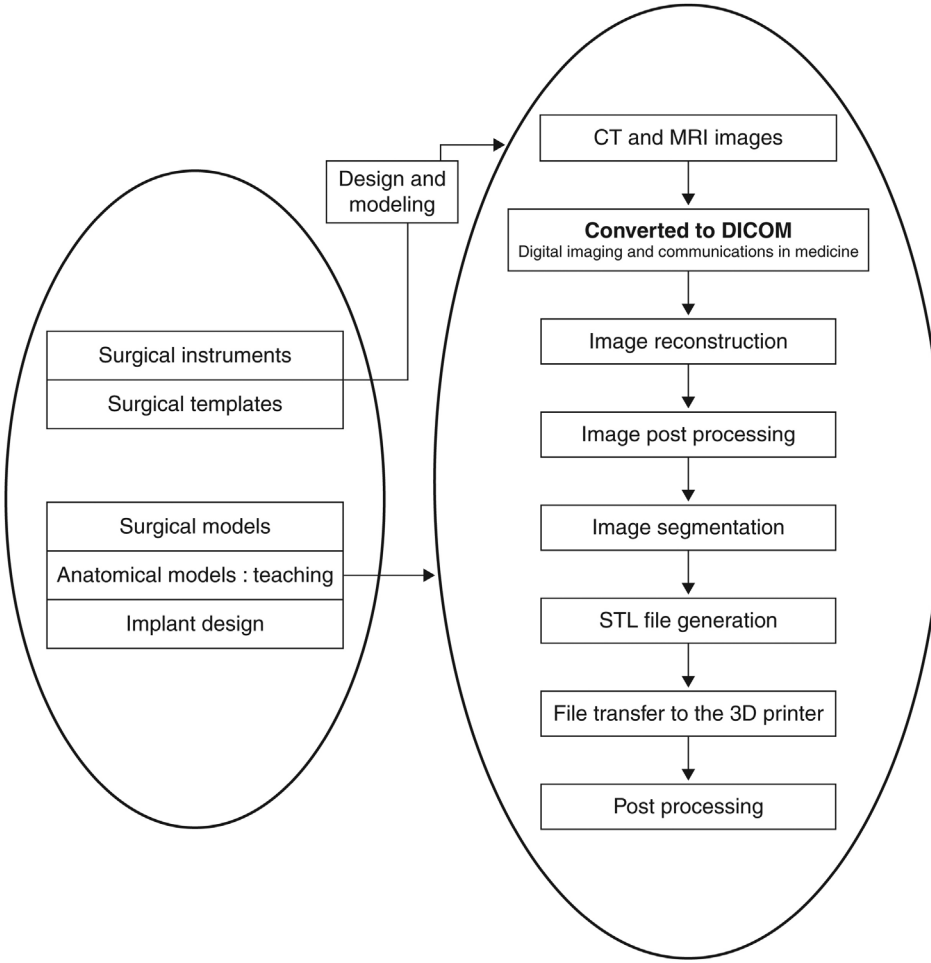

Figure 12.6 Schematic showing the steps involved in preoperative planning of surgery.
Source: Reproduced with permission from Rath and Sankar [67].

phenotype which can greatly differ from humans to animals [69]. 3D printing technology can be utilized for making realistic *in vitro* disease models due to its potential to mimic the actual cellular arrangement of the native human tissue or organ [70]. Patient specific 3D printed congenital heart disease (CHD) model are widely being used in the area of cardiology and cardiac surgery. Tactile experience offered by the 3D printed models is a major advantage over the traditional image visualization [71]. Fig. 12.7 gives the steps in involved the fabrication of a typical CHD model.

One of the major challenges in the fabrication of a disease model points the complex interactions between different organs during a chronic disease process. Mimicking these complex interactions/cross talking is a major challenge in the development of an ideal 3D disease model [72]. Other key challenge is to integrate the circulating

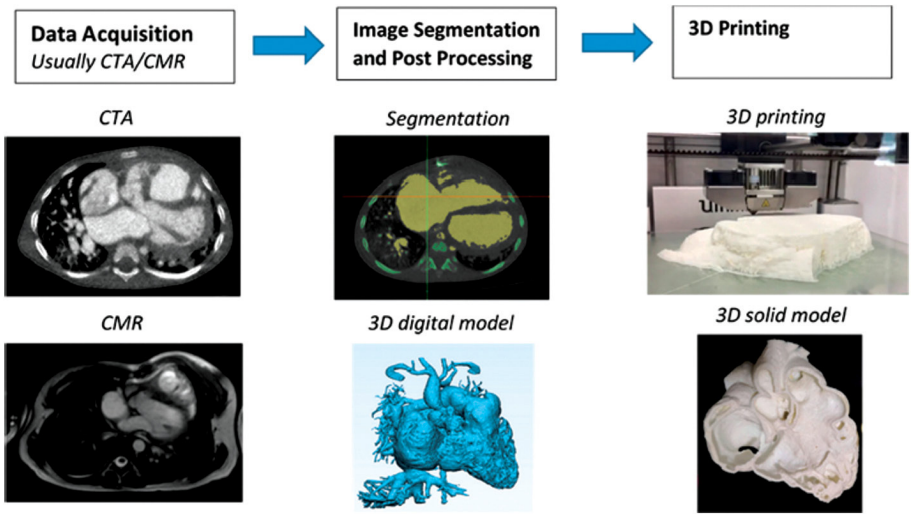

Figure 12.7 Steps involved in fabrication of 3D printed heart models. CTA, computed tomography angiography; CMR, cardiac magnetic resonance; 3D, three-dimensional. *Source*: Reproduced with permission from Sun et al. [71].

immune cells and the inflammatory responses into engineered in vitro disease models. Immune elements play a critical role in the disease progression so are the important features in the development of an *in vitro* disease model. Such models can be developed by using organ-on-chip device technology [72] (Table 12.1).

Table 12.1 Recent in vitro disease models with the methodology used, cells utilized, and types of diseases models.

Organ	Methodology used to fabricate	Cell types used	Types of human diseases modeled
Heart	Cells suspended in fibrinogen and 10 % Matrigel, mixed briefly with thrombin, and pipetted into rectangular agarose casting molds of 12 × 3 × 4 mm in a 24-well plate.	Mice Cardiomyocytes	Homozygous and heterozygous hypertrophic cardiomyopathy.
	Dry Gelfoam collagen sponges as scaffolds, seeding them with cells in Matrigel.	Neonatal rat ventricular myocytes.	Diabetic myocardium, Developed to study hypoxia in cancer.
	Cells-in-gels-in-paper (CiGiP) approach, seeded with cells suspended in Matrigel.	Neonatal rat ventricular myocytes.	To study asthma-associated matrix remodeling.

Table 12.1 Recent in vitro disease models with the methodology used, cells utilized, and types of diseases models. (*Cont.*)

Organ	Methodology used to fabricate	Cell types used	Types of human diseases modeled
Lung	Type I collagen matrix in Transwell device.	1) Human fetal lung fibroblasts and human airway epithelium.	1) To analyze how ECM reorganization affects differentiation of cells in the airway wall, a central hallmark of asthma.
	ECM gels to develop a model of matrix stiffness-induced fibroblast differentiation.	2) Human lung primary cells, lung fibroblasts, airway smooth muscle cells	2) To investigate individual components of airway remodeling such as subepithelial fibrosis, smooth muscle hyperplasia and hypertrophy, and epithelial cell metaplasia.
	Air – liquid interface in Transwell inserts. Agarose gels with high-aspect ratio nanomaterials, such as carbon nanotubes. Human lung-on-a-chip microfluidic device	Human airway epithelium from COPD patients. Murine macrophages. Human lung alveolar epithelial cells and pulmonary capillary endothelial cells.	Goblet cell hyperplasia, The effect of cigarette smoke on the conducting airways, Squamous cell metaplasia. Recapitulate organ level physiology of the lung, inflammation due to lung infection and model of pulmonary edema.
Intestine	Human gut-on-a-chip Microbiome into 3D cultures by using microbead-based rotation chambers (termed microgravity cultures) or organoids. 3D organoid cultures leverage ECM or synthetic polymer hydrogels as scaffolds to support the growth and differentiation of cells.	Human intestinal epithelial (Caco-2) cell line. Human epithelial cells human intestinal tissues derived from iPSCs	To study the etiology and mechanisms underlying intestinal diseases, such as IBD Used in a model of norovirus infection, which causes diarrheal diseases in humans. Rotavirus infection and host – parasite interactions
Liver	A multiwell system with different types of cells in coculture.	Hepatocytes and 3T3 fibroblast	Used to model different types of liver diseases.

(*Continued*)

Table 12.1 Recent in vitro disease models with the methodology used, cells utilized, and types of diseases models. (*Cont.*)

Organ	Methodology used to fabricate	Cell types used	Types of human diseases modeled
Kidney	Microfluidic kidney-on-a-chip device. Cells cultured in type I collagen gels. A cylindrical microfluidic device was created with an inner diameter of ~400 μm and the microchannel was coated with a layer of glass using a sol-gel method and then coated with fibronectin.	Primary human kidney proximal tubule epithelial cells. Primary kidney epithelial cells isolated from cysts of autosomal dominant PKD (ADPKD) patients. Human proximal tubular (HK-2) cells	Recapitulate responses to toxic drugs *in vitro*. Human polycystic kidney disease (PKD).
Ovary	Microbricated PDMS molds	Ovarian theca and granulosa cells	The in vitro regulation of ovarian follicle development. Simulate human ovarian physiology and model for the maturation of human oocytes
Skeletal muscle	Micropatterned myotubes on a fibrin gel sheet combined with a microelectrode array chip *in vitro* three-dimensional (3D) type I collagen matrices under uniaxial tension. *In vitro* three-dimensional (3D) type I collagen matrices under uniaxial tension Genetic homolog of DMD, where tissue engineered in 96-microwell plates into 3-dimensional muscle constructs with parallel arrays of striated muscle fibers.	C2C12 Multiple population doubled (MPD) murine myoblasts Dystrophic murine myoblasts	Closely mimics type 2 diabetes. Constructs that model aged phenotypes or muscle degeneration with age Model of Duchenne muscular dystrophy

Table 12.1 Recent in vitro disease models with the methodology used, cells utilized, and types of diseases models. (*Cont.*)

Organ	Methodology used to fabricate	Cell types used	Types of human diseases modeled
'Bone marrow and hematopoiesis	Cultured within a type I collagen gel.	Human proximal tubular epithelial cells.	The acute tubular injury produced by cisplatin.
Vascular system	Coating the flow chamber surface with collagen, plaque material, and thrombus material (such as von Willebrand factor and fibrinogen) Microfluidic channel engineered with a geometric (concave semicircular) constriction to mimic a stenotic atherosclerotic vessel.	Human vascular Endothelium Human endothelial cells	In vitro models of atherosclerosis. Modeling of the atherosclerotic plaque.

Source: Reproduced with permission from Chameettachal and Pati [72].

12.8 High resolution 3D printing to improve healthcare

To enhance the precision and to broaden the application niche, several high-resolution 3D printing techniques have been introduced to address the challenges faced by 3D printing. One major challenge is being the fabrication of complex structures with high precision. Introduction of high-resolution 3D printing has shown applicability in the area of tissue engineering [73], nanoelectronics [74], photovoltaics [75] and orthopedics [76].

12.8.1 Different types of high-resolution 3D printing techniques

Most of the high-resolution 3D printing methods currently in use fall under direct write (DW) techniques. DW can be defined as "any technology that can create two or three dimensional functional structures directly onto flat or conformal surfaces in complex shapes, without any tooling or masking" [77,78].

12.8.1.1 Direct write printing (DW)

During DW printing process rationally formulated inks are extruded through a custom-made microscale nozzle using compressed air. Distance between nozzle and the

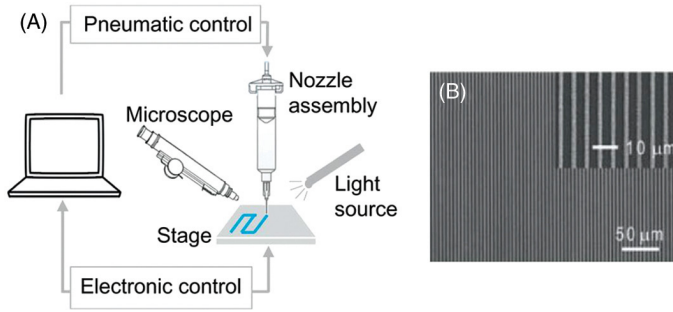

Figure 12.8 (A) Printer set up for DW printing and ink deposition through a customized nozzle, and (B) an example of high-resolution printed electrode array.
Source: (Reproduced with permission from Fang et al. [79].

substrate is adjusted to minimum so that when ink extrudes from the nizzle it forms a liquid bridge or a capillary bridge [79]. Printing resolution is controlled by playing with the diameter of the nozzle which can range from sub-micrometers to 500 µm. Technique can be used with the broad range of inks namely colloidal suspensions, fugitive organic inks, sol–gels, polymer melts, etc. [80]. Fig. 12.8 represents a typical set up for a DW printing.

12.8.1.2 Electrohydrodynamic printing (EHD)

EHD provides high resolution in comparison to the standard printing techniques , for example, inkjet printing [81]. High resolution is achieved by applying an electrical filed between miniature nozzle (which has an inner diameter between 100 nm to few µm) and substrate [82]. Because of assisting electrical field lines, EHD enables an accurate droplet placement which can be exploited to print high resolution 2D and 3D structures.

12.8.1.3 3D direct laser writing (DLW)

DLW technique offers a means to print 3D structures using photosensitive materials at a resolution as low as sub 100nm [83]. Typical DLW involves focusing on ultra-fast laser beam into a small volume (voxel) inside a photosensitive resin to initiate a local photopolymerization. Fig. 12.9 represents a standard set up for controlled 3D cell culture studies.

12.8.1.4 Focused ion beam (FIB)

FIB offers microscale to nanoscale level scaffolding capabilities. It offers two-way functionality, with which, it can remove material through sputtering or micro-machining, and it can add material through ion-induced decomposition or deposition. FIB provides a high resolution patterning as particles used in this technique are heavy charged ions so they move in straighter path in comparison to the electrons in electron beam deposition [84].

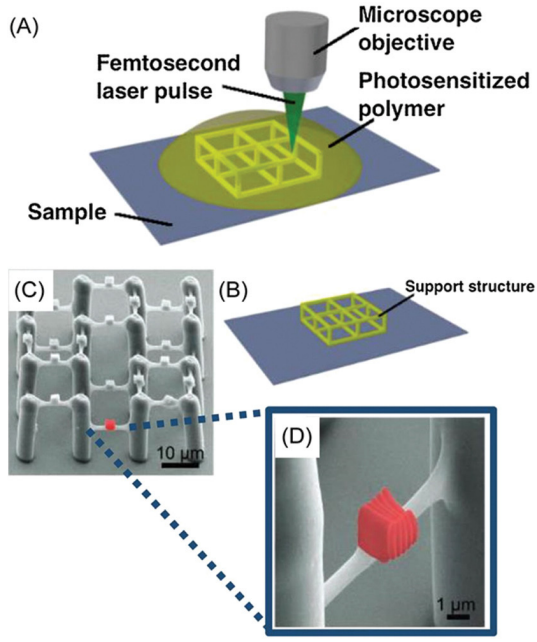

Figure 12.9 DLW for controlled 3D cell culture studies. (A) DLW printing, (B) Resulting scaffold structure after removing the nonpolymerized monomer, (C) DLW of 3D frameworks consisting of protein-repellent PEG-DA, (D) Photoresist Ormocomp cubes precisely placed. *Source*: Reproduced with permission from Fang et al. [79].

12.8.2 Applications of high-resolution 3D printing in medicine and diagnostics

High resolution 3D printing has demonstrated immense potential in the area of healthcare sensors and implantable devices. Muth et al developed an embedded 3D printing approach to fabricate a soft mechanical strain sensor [85]. Xu et al used high resolution 3D printing to fabricate a heart model integrated with network of sensors and electrodes to monitor heart activity. Further developments with this device could potentially replace the current pacemakers [86]. It is expected that as the area of high resolution advances it can be applied for the fabrication of personalized implantable devices those can be customized according to patients needs thus playing a key role in the area of personalized medicine [79].

12.9 Applications of 3D printing in regenerative medicine

3D printing has made a huge impact on array of research fields from biotechnology to diagnostics. One particular area which has made a significant progress by the advent of 3D printing is regenerative medicine [87]. Regenerative medicine is an emerging

area which aims to replace or regenerate the diseased organ or tissue. Goal of regenerative medicine or tissue engineering is to recapitulate the anatomical, biochemical, and functional components of different human tissues or organs to fabricate an ideal tissue or organ which can be used for replacement in patients which have no treatment option other than transplantation. Conventionally tissue mimics were fabricated using the different scaffold fabrication techniques namely hydrogelation, cryogelation, salt leaching, etc. [88]. But in all these methods as processes are not controlled, it is very difficult to achieve precision and reproducibility. Other major disadvantage with the conventional scaffolding techniques is that they are not able to mimic the complexity of the native tissue or organ. To answer these limitations, 3D printing has emerged as a potential tool which is capable of adding up the complexity of a living tissue with high precision. Advances in the area of 3D printing have led to printing of 3D live tissue where hydrogel is deposited with the live cells to form 3D tissue structures [89,90]. Following section discusses the scope and success of 3D printing in the area of regenerative medicine.

12.9.1 Skin

Skin tissue engineering is most advanced area and has the largest number of skin substitutes available commercially, for example, Biobran, Alloderm, Dermagraft, and Apligraft. Although these skin substitutes have been clinically very successful they don't fully mimic skin as they lack the intricate vasculature, hair follicles, pigments, and glands [87]. Lee et al. developed a 3D printed skin graft using different dispensers those were independently operated by electromechanical valve. By varying the dispensed droplets, liquid material and air pressure different cell types such as keratinocytes and fibroblasts were printed in layer-by-layer manner [91]. Furthermore, Koch et al. fabricated 3D skin grafts by printing fibroblasts and keratinocytes in collagen using laser-assisted bioprinting with the formation of basement membrane and intercellular junction [92]. Michael et al fabricated a 3D printed tissue construct with printed keratinocyte forming a multilayered epidermis, *in vivo* studies indicated the potential of this 3D graft for the repair of full thickness skin wounds [93]. Because of the wide acceptability of the non-3Dprinted skin substitutes, major challenge of 3D printing skin grafts is that they should be able to outperform the former in preclinical and clinical studies. 3D printed skin substitutes have been explored extensively in cosmetics industry. L'Oreal which is a major cosmetic brand has partnered with Poietis a French biotech company to print skin with hair follicles for cosmetic testing [3].

12.9.2 Bone and cartilage

Aim of 3D-bioprinting is the fabrication of a bone graft which mimics the functionality of the native tissue including high mechanical strength, native cellular microenvironment, etc. [94] (105). For regeneration, different cell types are being used but human mesenchymal stem cells (MSC's) have shown an excellent capacity to differentiate into bone, and therefore are categorized as a potential cell type for bone regeneration. In addition to an ideal cell source other important parameters, those can influence

the success of a 3D printed scaffold is the type of biomaterials, soluble biomolecules and cell-cell interaction [87]. Different research groups have tried different materials to print an ideal bone graft Duarte et al. reported that addition of agarose to collagen can significantly improve mechanical properties of the scaffold and at the same time promote osteogenic differentiation of human MSC's [95]. Next important factor is the differentiation or growth factors; those can be delivered in previously printed scaffold either on the surface or within the micro-porous channels. Incorporation of the growth factors has shown a substantial effect on then growth and differentiation of stem cells. An *in vivo* study with biphasic calcium phosphate (BCP) bone implants were fabricated using 3D printing enhanced bone formation in rats [96]. Several 3D printed biomaterials have shown substantial potential to enhance osteoinductivity, for example, 3D printed HA and tri-calcium phosphate (TCP) ceramic scaffolds have shown to improve bone regeneration by releasing calcium ions [97]. Addition of SrO and MgO in 3D printed TCP scaffolds has also shown early healing through accelerated osteogenesis in rat defect models [98,99]. Tissue engineering of cartilage tissue is encountering some major challenges, for example, lack of integration with the native tissue and mimicking the complex 3D zonal cartilage architecture. So, 3D bioprinting is being explored for the fabrication of an ideal scaffold which can answer the limitations of the conventional scaffold fabrication techniques. Recently, electrospum poly (caprolactone) fibers were altered with rabbit elastic chondrocytes using hybrid inkjet printing system. Results from this study indicated that 3D printed scaffold had same zonal organization as that of articular cartilage but lacked the integration properties [99,100]. Other concept which is gaining population for the regeneration of cartilage is "cell homing" by which cells migrate from the local tissue in response to a chemotactic response which can be growth factor or other type of stimulator. Cell homing is being used in the area of cartilage regeneration by the recruitment of the local cells under the influence of the growth factors. 3D printed scaffolds with a spatial organization were fabricated to deliver TGFβ3. Results indicated that TGFβ3 infused biscaffold when implanted *in vivo* was fully covered with hyaline cartilage with defined blood vessels and exhibited similarity to the native tissue [101]. These and other similar studies suggest that homing of multiple cell population may be a viable option to cell transplantation for cartilage in clinical applications [102]. There are no commercial 3D printed products for bone and cartilage available in market yet, but some of the products are in clinical trial phase 1 or 2 [87].

12.9.3 Cardiac tissue

3D bioprinting of cardiac tissue is a complex problem as mimicking the native tissue architecture is challenging to achieve. But with the increasing statics of cardiac related ailments worldwide there is a huge demand for an alternative to heart transplantations. Several animal studies have already indicated that 3D bioprinted cardiac patches have ability to reduce the fibrosis, hypertrophy and infarct extension [103–108]. Different types of bioinks from gelatin, collagen, alginate, etc. are being explored for cardiovascular applications [109,110]. A bioink made from decellularized ECM which represents a true mimic to the cardiac tissue was investigated and was shown to exhibit a

significant effect on stem cell differentiation which was not observed with other types of bioinks [111,112]. From the cell type perspective different cell types such as ESC's, iPSC's, and MSC'S have shown application in 3D bioprinting. Fig. 12.10 illustrates different types of stem cells used for 3D bioprinting of the cardiac tissue.

Cardiovascular 3D bioprinting can be accomplished in following two ways:

12.9.3.1 3D bioprinting cardiac patches

Fabrication of 3D bioprinted cardiac patches is the most promising method for cardiac tissue regeneration. Major advantages here are a complete control over the structure and design of the construct and the incorporation of multiple cell types to mimic the native tissue architecture [87]. Various *in vivo* studies with different cell types have proven initially successful. Wang et al. designed 3D bioprinted cardiac patch using fibrin-based bioink on a hydrogel contract [113]. In one of the *in vivo* studies alginate and PEG-fibrinogen patches with HUVEC's and iPSC-derived cardiomyocytes have shown integration with the host tissue together with the formation of vasculature [114]. "Biomaterial-free cardiac patches" printed as cellular aggregates have also been explored for cardiac tissue regeneration. In 2017 Ong et al. used human iPSC derived cardiomyocytes, fibroblasts, and endothelial cells in the form of an aggregate or cardiac spheroid. Spheroid exhibited vascularization and integration with the native

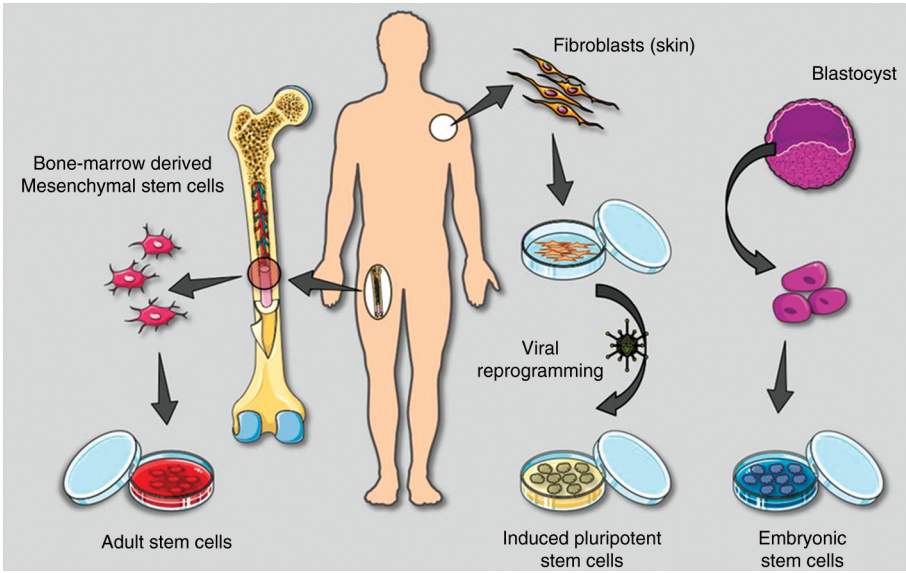

Figure 12.10 Stem cells for 3D bioprinting in cardiovascular tissue repair: MSCs harvested from the patient's bone marrow are developed into adult stem cells. Fibroblasts harvested from the patient's skin are reprogrammed *in vitro* to produce iPSCs. ESCs harvested from a blastocyst can differentiate into any cell type in the body.
Source: Reproduced with permission from Loai et al. [87].

rat myocardium indicating the potential of biomaterial free 3D printed cardiac patch technology [115].

12.9.3.2 3D bioprinting cardiac valves

For patients with valve diseases namely valvular stenosis, valvular prolapse, regurgitation,etc., valve replacement surgery is the only option, but it has its own limitations and associated risks. 3D bioprinting has been used for printing of valves from different biomaterials and cell types. Duan et al. used extrusion-based bioprinting to construct tri-leaflet heart valve conduit made from hyaluronic acid and gelatin seeded with human aortic valve interstitial cells [116]. In a follow-up study Duan et al. 3D bioprinted living aortic valve conduct using alginate and gelatin hydrogels seeded with aortic root sinus smooth muscle cells and aortic valve interstitial cells. Construct exhibited good mechanical integrity and high viability [117]. Furthermore, research must focus on exploring newer biomaterials, cell types, and growth factors, which are conducive for regeneration of the cardiac tissue. In conclusion, bioprinting of cardiac tissue may be a viable treatment option for the patients with heart failure, valve diseases, or other related cardiac aliments.

12.9.4 Nervous system (PNS and CNS)

Applications of 3D printing for the regeneration of CNS and PNS can be categorized into two different approaches:

1. *in vitro* models of the nervous system
2. scaffolds for nervous system tissue repair

12.9.4.1 In vitro models of the nervous system

Recently Gu et al. created a bioink containing human neural stem cells (hNSC's) and allowed the cells to be printed in a 3D structure. By printing hNSC's in 3D structure, it was observed that genes associated with differentiated neural cells were upregulated when compared to the 2D cultures [118]. In the follow-up study, authors demonstrated the ability to print human iPSC's into 3D structures either maintain iPSC phenotype or differentiated into neural cells [119]. Results for these studies indicate that creating a 3D environment for iPSC's and hNSC's help them to differentiate efficiently in comparison to the 2D culturing. Creating a 3D layout also helps to mimic the native tissue complexity.

12.9.4.2 Scaffolds for nervous system tissue regeneration

Flexibility of 3D bioprinting for creating a demand on fabrication makes it an appealing option for PNS and CNS repair. AS bioprinting practically eliminates the need of surgically harvesting of the nerve graft for nerve repair as it can create grafts of any size and shape. Different research groups are working toward the development of 3D printed peripheral conduits for clinical trials. One potential approach to do this is to use spheroids from cells namely MSC's, Schwann cells, neural rat cells, etc. [120–123].

Spheroids can be used as a bioink for nerve guidance conduits [124]. Zhang et al. used MSC's for the formation of spheroids those were further differentiated into Schwann cells or neural cells to form cellular nerve graft. On both functional and histological assays 3D printed grafts performed at par with the autografts [121,123]. To further in this area, there should be more extensive research focused on the comparison of 3D printed graft to the autografts to move the 3D printed grafts to the clinical settings. Fig. 12.11 represents the stages of development for 3D printing of brain and nerve tissues.

12.9.5 Renal tissue

Approximately 850 million people worldwide have some form of kidney ailment. In case of chronic kidney related malfunctions transplantation is the most appropriate approach for treatment. But with limited donors' available researchers and clinicians are looking for other alternative treatment regimens and 3D bioprinting is one of the most promising options. Bioprinting whole human kidney is an arduous task but 3D printing a kidney tissue models is a practical option and is important in drug development studies [87]. Renal tissue regeneration using 3D bioprinted can be performed in following two ways:

12.9.5.1 3D bioprinting in vitro renal tissue models

Most of the *in vitro* renal models are focused on recreating proximal tubule (PT) cell function. Homan et al. fabricated 3D bioprinted convoluted human renal PT and also demonstrated that cyclosporin which is a potent nephrotoxin affected the epithelium in dose dependent manner [5]. Fig. 12.12 illustrates different steps of fabricating 3D convoluted, perfused, proximal tubules.

Additionally, 3D bioprinted renal models can also play a significant role in the development of a disease models to study kidney functions and drug toxicity. Lin et al.

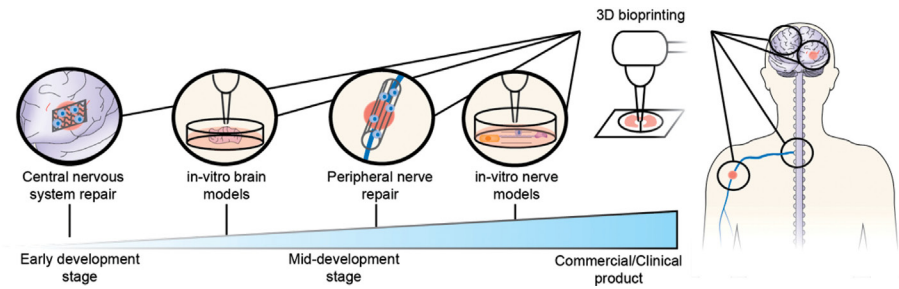

Figure 12.11 Different stages of development for 3D bioprinting of brain and nerve tissues. 3D bioprinting has been investigated for multiple nervous system tissue regeneration applications. On the left, scaffolds for central nervous system repair are still at a very early stage in development, as are 3D printed *in vitro* brain models. 3D printed scaffolds for peripheral nerve repair are slightly further in development, as are *in vitro* models of peripheral nerves. *Source*: Reproduced with permission from Loai et al. [87].

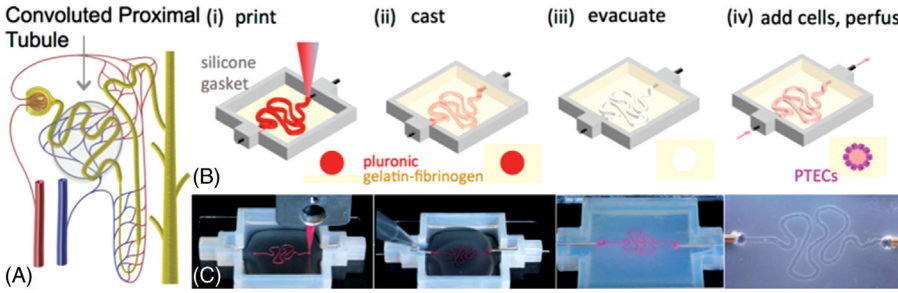

Figure 12.12 ***In vitro*** **renal model of the convoluted proximal tubule.** The different steps of fabricating 3D convoluted, perfused proximal tubules (PT). (A) Schematic of a nephron. (B,C) corresponding schematics and images of different steps in the fabrication. A fugitive ink is printed on a gelatin-fibrinogen ECM (i). Additional ECM is cast around the printed feature (ii). The fugitive ink is evacuated to create an open tubule (iii). PT endothelial cells (PTEC) are seeded within the tubule and perfused for long time periods via an external peristaltic pump (iv). *Source*: Reproduced with permission from Loai et al. [87].

3D bioprinted a model to investigate albumin uptake and glucose reabsorption, such models can act as valuable platform for future renal physiology and pharmacological studies [125].

12.9.5.2 3D bioprinting renal tissue for regeneration

Kidney is one of the complex human organs; it has over 30 different cell types, and very intricate internal compartmentalization. Considering all these facts, bioprinting a renal tissue is a grueling task to accomplish. There are no reports available on the bioprinting of renal tissue at organ level for regeneration [87]. Further development in the area of biomaterials, 3D bioprinting, bioink formulations may be provide beneficial in getting a better insight into 3D bioprinting of a renal tissue on organ scale (Fig. 12.13).

12.9.6 Liver

Liver is a vital organ of human body involved in metabolism, detoxification, and homeostasis. According to research reports, there are high incidence of liver mortality and morbidity induced by drugs and pharmaceutical products. Many pharmaceutical products fail at the clinical trial level just because they can induce high liver toxicity [126–128]. Therefore goal of 3D bioprinting is to replicate the intricate architecture of the liver and also to aid in the development of a disease model for drug testing [126,129].

12.9.6.1 3D bioprinting in vitro liver tissue models

In vitro cell line models are largely limited to 2D which does not mimic 3D native architecture posing a major limitation to development of a strategy for regenerative purposes. Ma et al. proposed a 3D printed tri-culture model made from human iPSC-derived hematopoietic progenitor cells (iPSC-HPSC's), HUVEC's and adipose-derived

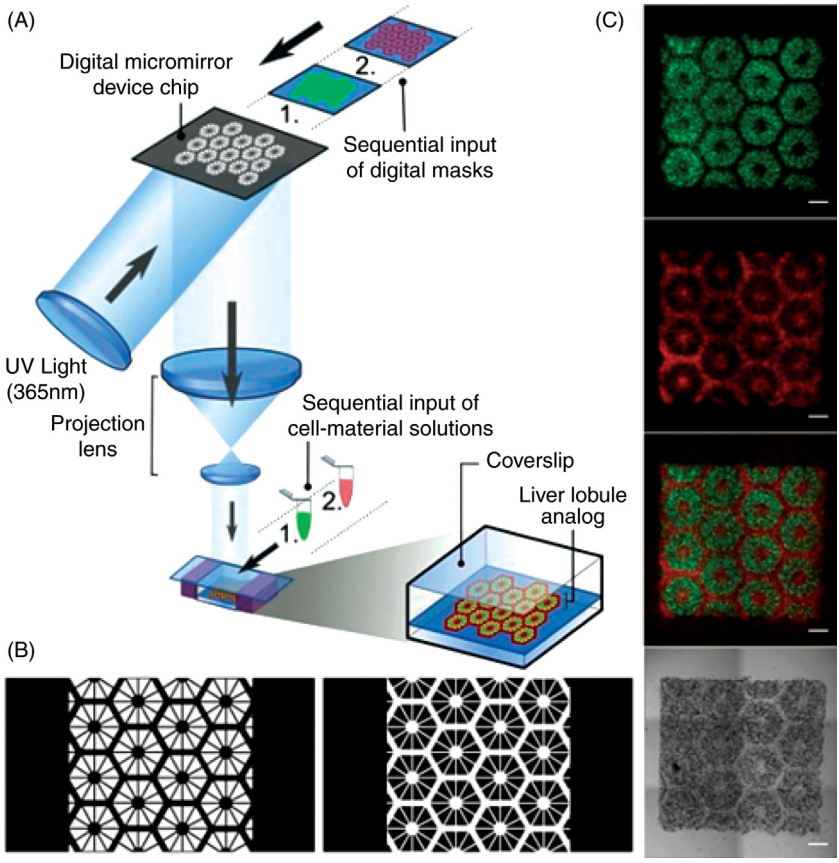

Figure 12.13 3D bioprinting of hydrogel based hepatic construct. (A) Schematic of a two-step 3D bioprinting approach in which hiPSC-HPCs were patterned by a digital mask, followed by patterning via a second mask. (B) Grayscale digital masks for polymerizing lobule structure (left) and vascular structure (right). (C) Fluorescent images (5 ×) show patterns of fluorescently labeled hiPSC-HPCs (green) and supporting cells (red) on day 0. (Scale bars, 500 µm.)
Source: Reproduced with permission from Loai et al. [87].

stem cells embedded in a bioprinted hydrogel system. Fabricated 3D printed model exhibited improved morphological organization and enhanced metabolic product secretion. Figure 12.13 gives the details of the fabricated system.

12.9.6.2 3D bioprinting liver tissue constructs for regeneration

There are few research reports on the fabrication of 3D bioprinted liver tissue constructs for regeneration purposes. Faulker-Jones et al. bioprinted human iPSC-derived hepatocyte like cells and human ECS's on alginate hydrogels. Bioprinted cells exhibited morphological similarity to the hepatocytes and also maintained functionality of

secreting albumin [29]. According to the most recent research report published in year 2019 continuous 3D bioprinted technique was used to work with decellularized tissue specific ECM bioink. Human iPSC-derived hepatocytes were shown to maintain high viability and functionality [130].

12.9.7 Future directions and challenges

3D bioprinting has shown huge possibility in the area of regenerative medicine and is the future hope for the development of novel therapies. For the efficient translation of 3D bioprinting to clinical settings following challenges should be addressed.

12.9.7.1 Biological issues

Major biological issues that 3D printing is facing now are limited oxygen diffusion, cell perfusion, cell migration, and vascularization through the printed organ or a tissue. These limitations can be addressed by designing a model which has an optimum pore size, porosity, and pore size which can favor vascularization [131].

12.9.7.2 Engineering issues

These issues include efficiency, reproducibility, practicality, process biocompatibility, etc. Developments are underway to improvise pre-processing, processing, and post processing steps. For example, for pre-processing intelligent hydrogels, bioinks and biomaterials are being explored to make the whole process more controlled [132]. For post-processing steps, strategies are being developed to directly transplant the bioprinted tissue in vivo so that maturation can occur in the native environment [133].

12.9.7.3 Cost

3D bioprinting being a novel fabrication technology is very expensive. There is a room to develop or modify the processes to make this technique economically feasible as a regular treatment method [131].

12.9.7.4 Regulatory issues

3D printing being a novel treatment method does not have any set of regulatory and safety guidelines. Thorough and strict regulatory guidelines should be imposed to ensure the safety and reproducibility of the 3D printed organs and tissues [131].

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Part 3

Future Technology and Regulation

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3D printing future perspective in medicine

13

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Chapter outline

The prospect of producing whole organs for transplant has driven the exponential growth of 3D bioprinting in the recent times. While it is still in the realm of scientific fantasy to mass produce custom designed organs, there are several limitations to that need to be overcome before 3D printed organs could be available [1]. 3D printing utilizes the advances in the fields of cells technologies, tissue engineering, material processing, computer-aided designing, and manufacturing practices. Present strategies that are used for 3D bioprinting are (1) Inkjet-based bioink [2,3], (2) extrusion-b, (3) laser assisted, (4) acoustic bioprinters, (5) stereolithography based, and (6) magnetic bioprinting. Almost all of these techniques work extremely well for creating small 3D printed tissues. Because of this 3D printing has already made a considerable impact on several disciplines [4]. The development in bioprinting has enabled the engineering transplantable tissues including multilayered skin graft, bone, tracheal splints, vascular grafts, and heart tissue [5]. This technique has come long way with the range of bioinks design for different application expanding rapidly. Depending upon the property of the scaffold of hydrogel needed this technology can adapt, change and produce materials in intrinsic detailing that is impossible to achieve with any other technique presently, The bioprinted organ development is a multilayered processed in which cell proliferation, material degradation, and remodeling of the matrix must occur simultaneously. It is possible to use mathematical algorithms to predict this development and currently the major area of focus in bioprinting arena. If there is a program that can predict cellular behavior to the engineered matrix it could open new doors of possibility in which patients can be treated on specific case basis instead of being treated with general implant that has been mass produced. Besides tissue engineering 3D printing has major role to play in pharmaceutical industries too [6].

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13.1 3D printing in drug discovery

Drug discovery is an expensive and a time-consuming process, often spanning about 12–15 years costing more than $1 billion. Discovering new drugs consists of two distinct phases, preclinical and clinical phase. High-throughput screening (HTS) a common technique that is used in the preclinical phase for identifying a new lead molecule [7]. Starting with thousands of molecules, only a handful of molecules survive the rigorous testing to become lead molecules. Most of the testing in high-throughput screening is performed on 2D monolayer cultures grown in polystyrene or plastic plates. However, the 2D cultures do not accurately represent the tissue as tissues are complex 3D structures composed of cell layered with surrounding extracellular matrix. With the advances in cell culture technologies which include organoids and 3D cultures, there has been a progressive shift from the traditional 2D culture to 3D culture in high-throughput screening. 3D cultures have also proven useful in evaluating the relatively newly developing drug delivery systems including but not limited to nanoparticles and triggered release drug delivery systems.

Despite the advantages of 3D for high-throughput screening, only a few 3D culture models are actively being used in the industrial settings. There are several limitations to using 3D cultures. These include a higher cost to set up, heterogeneity of the different 3D printed cultures including differences in extracellular matrices [8], variability and difficult to implement automation [9]. The most important limitation is the lack of vascularization in the 3D cultures. The cells in the inner layer tends to rely on simple diffusion for oxygen and nutrients, thereby limiting the size of the 3D culture. An increase in the size of these cultures would create differences in the way the cells react to the availability of the drug. Several groups have been trying to address this problem but with limited success. Another important aspect is the lack of immune system in the printed 3D cultures. Recently some investigators have been incorporating immune like cells to address this issue. The possibility to incorporating several different types of cells to mimic tissue types has been explored with success.

Irrespective of the fact that the 3D printing is yet to be widely adopted and more expensive than the conventional high-throughput screen and drug discovery process, the potential of developing novel methodologies such as “organ-on-chip” poses a unique opportunity of possibility of replacing ex vivo organs cultures. Despite the limitations, 3D cultures have the potential to decrease the time and resources for preclinical drug discovery process.

Bioprinting can be used in preclinical phase of discovery and development of drug. After validation and selection of HTS target which is undertaken in target-to-hit stage during which there are 10^5–10^6 possible compounds are tested for its ability to bind with specific target. Its primary goal is to identify as many specific chemical hits as possible. Depending upon the target and assay, the output of this test is typically in few 1000s compounds that can produce assay signals. At this stage the confirm hits are divided into chemical series and each of this hit is evaluated with respect to its potency, comprehensive properties (cost and scalability) and physiochemical nature. Compounds that survive the triage of testing enters what's known as hit-to-lead stage that evaluate the in vitro efficacy and predictive in vivo toxicity of each of the hits.

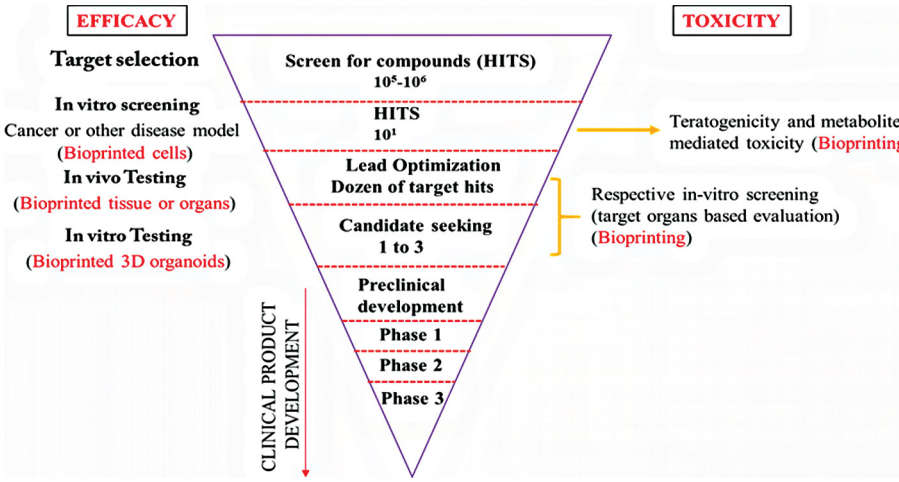

Figure 13.1 Future prospective of 3D bioprinting use in drug discovery and clinical drug development.

Libraries developed provides substrates for next level in which structure–activity relationship is test for target-binding efficiency. Most important aspect is cell-based target modulation testing in which bioprinting can play crucial role. Although bioprinter can be used in every step of drug testing due to its versatile process and can cut down the in vitro cell testing cost, it is utmost important to develop robust in vitro screening process to achieve commercially viable drug discovery and development process (Fig. 13.1).

13.2 3D printing in cancer treatment

There is major interest in using of 3D models that can replicate cellular response more precisely especially in case of cancer treatment that can overcome limitation of conventional monolayer culture. One main reason for this is that 3D models can be used for rapid screening of drugs which reduces the need for expensive in vivo experiments. 3D cancer models especially spheroids cultures have extensively contributed to photodynamic research by allowing to examine the uptake and the therapeutical efficacy of different photosensitisers. Photodynamic therapy heavily depends upon the hypoxia conditions that are found in solids tumors which cannot be replicated in monolayer cultures. Penetration of the oxygen in 2D and 3D system and how 3D spheroids can replicate solid tumor that grows in vivo has been extensively studied. Using microfluidic device or bioprinter to engineer 3D cancer tissue models holds more advantages than traditional cell culture techniques as it allows replication of medium and drug perfusion in scenario closer to the in vivo system [10]. Radiotherapy aims to delivery precise and curable radiation dose to tumor site while sparing the surrounding tissue. This precision is achieved by accurate conformal delivery of the ionizing radiation [11]. 3D printing the affected tissues and then using it to study

Figure 13.2 Experimental setup of phantom measurement for airway cancer treatment. *Source*: Reproduced with permission from ref [11].

and standardize radiation dosage before applying to the patient can decrease the risk associated with radiation toxicity that can be life threatening in some case. Utilizing 3D bioprinter to replicate tumor by using patient scan data could allow more targeted delivery of drugs to the tumor while sparing the surrounding healthy tissue is one of the most promising application of this technique (Fig. 13.2).

13.3 3D future for tissue engineering

Capability of 3D printer to accurately position cells and engineering an intrinsic network of scaffold is unachievable by any other conventional tissue engineering techniques. It has ability to places single cells per droplet and has ability to make 3D architecture that can closely mimic the in vivo organs and tissues. Extrusion bioprinting has poor cell survivability but can be used large 3D structure whereas laser-based bioprinting has higher cell survivability but cannot be used for making large structure hence combining both this technique would result in obtaining scaffold with intrinsic detailing and high cell viability which is ideal combination for tissue engineering approach. One of the biggest challenges of tissue engineering that can be overcome using 3D printing is vascularization of the scaffold. Using laser-based printing along with inkjet could enable vasculature to be printed onto the scaffold [1]. It is one of the biggest challenges involving translating bioprinted scaffold from lab to functional tissue is creation of vascular networks. Without suitable conduits for nutrient diffusion and waste disposal tissues with even minor complexity cannot survive and function in vitro. In the in vivo too these networks are essential for tissue growth beyond 100–200 μm, which is diffusion limit of oxygen. Hence, without proper vascular networks tissue engineered construct will have nutrient limitation that over period results in necrosis or incomplete tissue formation [12]. To have enough perfusion in bioprinted tissues, a vascular network must be present at early stage enough to prevent tissue death at developmental stage and to allow endothelium attachment and growth. As tissue develops vascular networks must perform all the normal roles that is involved in development including forming a selective barrier for nutrient transport and waste disposal along with participating in inflammatory reactions, maintaining homeostatic and perform coagulation functions. In order to overcome, the current challenges such

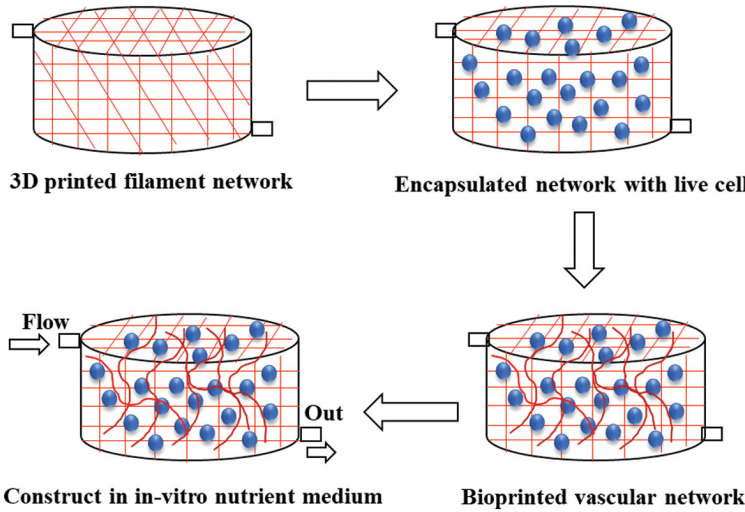

Figure 13.3 Schematic showing 3D printed scaffold with cells along with vascular network that allows preferential flow of nutrients and waste disposal.

as limitation of printing resolution and speed. Vascular network like capillaries can be extremely small as 3 μm in diameter, while the highest resolution that can be achieved by laser-based printing is of about 20 μm [13]. Even if printing resolution can be enhanced to certain degree achieving degree of complex network of capillaries that can be printed presently in limited and time consuming. The time is takes to print fine capillaries can compromise the cell viability. There are many challenges that needs to be overcome and presently many researchers are working on various solution such as incorporating angiogenic growth factors into the bioinks which can hire host vasculature growth following implantation of bioprinted constructs.

Since the advent of the 3D printing great progress has been made toward engineering functional tissue construct that can perform exact functions of the tissue its intended to replace. Despite challenge from the early period investigators have pushed through the bottleneck and proved 3D bioprinting is worthy of ongoing investigation. There is a need to involve multidisciplinary expertise in order to fulfill the clinical potential of 3D bioprinting, but future is indeed bright and it is poised to play crucial role in personalized regenerative medicine in near future (Fig. 13.3).

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Regulation and safety

14

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Chapter outline

Three-dimensional bioprinting faces significant regulatory and socio-economical challenges. The customization and complexity of additive bio-manufacturing (bio-AM) systems trigger the prerequisite assessment of the necessary rules in the domain of medical devices, tissues, cells, advanced therapies, pharmaceutical, chemical, blood, and food. The main regulatory challenge is in defining this process and categorizing the product associated with 3D bioprinting. Moreover, developments in this field might eventually, bring the current medical practices within the domain of the data ownership and protection of intellectual property rights (IPR) under pressure. 3D printing also raises serious legal challenges and risk related to storing and using extremely personal physical or medical information of patient who are the end user of the product. In order to create a customized medical artifact many individuals in a decentralized supply chain can have access to exclusive physical and medical details and/or images of the patient's body. This raises the issue of illegal sharing of these details among some communities like sharing of MP3 or video files which presents further threat to IP protection.

As 3D printer becomes more common and powerful with many independent service providers gaining access to highly sophisticated printers and manufacturing parts decentralization of production will increase and might potentially move to more private sector. This scenario can re-balance the knowledge sharing and manufacturing between private and public sectors. Safety regulations are most likely to be affected, might become outdated or irrelevant from the medical-ethical rules point of view. The informed consent, autonomy, quality, access to care and protection of vulnerable groups, clinical effectiveness each of these rules needs to be revisited and reformulated with 3D bioprinting on mind. This technique is expected to affect policies related to use of substances of human origin like tissues or cells, blood, and organs. The intrinsic play of materials like polymers and biological human derived cells with alternative manufacturing process can create an inequality in access to

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therapies and services. However, the most important aspect to remember is this technique first and foremost alleviate the shortage organ donors that would put an end to illegal trade of human organs. Furthermore, it allows using of patient's own cells meaning there is no issue of immune rejection of an organ hence, all the rules and regulations that need to be formulated should remember that the impact 3D bioprinting can have on human life.

14.1 Safety regulations

Safety constitutes a major regulatory challenge in 3D printing arena. In this area, safety primarily refers to the risk associated with medical procedures performed outside professional medical environments. Some side effects of bioprinting can be of concern which includes questions about the biomaterials, integration of manufactured tissue, biodegradation, neo-tissue formation that goes along the tissue formation. In the frame of 3D printing, many nonmedical (lifestyle) interventions have been performed in nonmedical setting that raises safety concern and needs to be addressed immediately. The intended grafting of living cells into human body has various risks for patient's health. New players in the field like DIY communities and hospitals that are decentralized medical economy, raises new challenges with regard to oversight of regulations. Safety and health are major concerns surrounding 3D printed due to widespread DIY practices in this field. Furthermore, the source of biomaterials, implant efficacy, unhealthy donor, and post-transplantation infections are cause of major safety issues. As 3D printed techniques and products remain untested clinical paradigm and as its based on placing live cells into human body there are risk that includes cancer and tumor formation, misgrafting, dislodgment, and migration of cells away from the site of injury.

With many bio-AM applications entering clinical trials phase, there are issues regarding the printed materials and actual printing process and side effects it can cause on human subjects. Mixing of nanoparticles could also pose long-term risks for implants and might require post-surgical monitoring. To ensure quality control at the early stage testing of donor tissue, procurement of materials and cells there is governing body in EU known as EU tissue and cell directives. However, crucial question remains when the product in both patient-matched and made on demand how much monitoring and test is required before the product reaches the consumer? Regulatory bodies have come up with rules that each laboratory needs to follow in order to get the product to the clinical trail phase; however, for time being major amount of 3D printing tissues and organs are in experimental scale and there might be some inherent safety issues that needs to be answered and major regulatory rules have to be set up to stop the exploitation of patient private data. However, let's not forget 3D printing is presently the only technique that can overcome organ donor shortag;e hence, the rules and regulation needs to be set up that allows the advancement of the 3D bioprinting along with protecting patient's privacy and holding stakeholders responsible whenever needed [1-3].

14.2 Security concerns

3D bioprinter is not like most traditional engineering technique, it heavily depends upon the personal data from patients and that raise potential concern not only to individuals but also collectively for the community. Some part of this concern comes from misusing the technique to engineer enhanced human tissue or organs by adding functions and using interbreed cells from human and animal to give individual competitive edge over others. These enhancement abilities can attract athletes and military personnel whose life depends upon their ability to perform better than rest of individuals. Even though so far, no such experiments have been conducted and highly unlikely to be performed by any clinician and researchers but could be possibility when technologies fall in wrong hands.

Furthermore, 3D printers have major cyber security and privacy challenges with important legal and business risk that needs immediate attention. Any dual use technique must be highly regularized, and any misstep can result in loss of public faith in technology. For instance, small controversy involving 3D printers was the blueprints of 3D printed guns readily available on Internet causes concern since no major expertise is required that with equipment that are easily available online could pave ways for production of weapons, bioweapons, or biohazard materials [4].

14.3 Legal classification of 3D printing

3D bioprinting like every other new technology has raised question about the exact legal nature more specifically about its specific categorization. Is bioprinter a machine or bioprinted materials “human organs”? The classification is crucial; however, since there are different rules for biological and nonbiological materials there is complication when it comes to 3D printer which when combined with 3D biomaterials on which live cells are cultured. It is further complicated as in reality 3D printed organs can be custom-made blending biological and nonbiological entities. No existing legal framework provides guidance's on use of substance of human origin with nonliving materials. Due to this combination along with process used for printing, current regulatory establishment need to reevaluate its regulation before 3D printed organs can be available for patients.

Presently main classification depends upon whether 3D printed device is mass-produced, or custom made in terms like is it produced on an individual level or small scale (in-house in hospitals). For now, these medical devices are treated as custom-made under Medical Devices Directives and has low regulatory burden than other counter-part medical devices. It is not subjected to ex ante oversight controls.

Recently implemented Medical device regulation (MDR) 2017/745 does not really regulate 3D printed device or process used for producing these devices. Under this regulation, 3D printed device continues to enjoy low regulation; however, manufacturer is required to strictly follow regulation in every step of medical device production. Any manufacturing should be prescribed by the healthcare professionals and prescription

must be part of paper work that is maintained while manufacturing the device. The patient for whom device is engineered must be the enduser and should meet the medical needs. That means there is no other solution to the patient's condition and medical device that is custom made for treatment must be used by patient and cannot be interchanged. These regulations raises furthermore questions like “can hospitals been host for industrial manufacturing process?”, “can the host deliver the device to other organization”? “can these in-house manufacture devices maintain and ensure quality of their products and remain compliant to present regulatory terms?” [5-7].

14.4 FDA regulatory and statutory terms

Statutory mission of FDA is simple and important “to promote and protect the public health”. This exclusive applied to the regulation implemented for “cellular and tissue-based products”. Hence FDA seems to be an appropriate agency to scrutinize, approve, and regulate 3D bioprinted organs when it's ready for human use. The federal food, drug and cosmetic act (FDCA) gives FDA authority to monitor and regulate any food, drug, and cosmetic entities. The available statutory definitions allow 3D bioprinted organs to be regulated as medical devices, biologics, drugs or any combination of these three items, which could subject it to various sets of regulations. These engineered and manufactured organs fall within the realm of FDCA governance, which requires greater consideration for suitable classification. Key is to determine whether 3D printed product is a drug, biologic, device, or cosmetic or combination of these components. Ultimately, this distinction depends upon the intended end use, mode of action, and all the ingredient used for its manufacturing.

FDCA clearly does not see 3D bioprinted organs as “medical device” as their definition of medical device clearly notes that device is an instrument, apparatus, machine, implements, in vitro agents, implants and contrivance or other related/similar articles, including components or parts which has same intended use as biologicals products or drugs. But “does not achieve its primary intended purpose through chemical actions within or on the body of man” … and does not depend upon metabolism to achieve its primary intended purposes. Hence, as body functions through the chemical actions and its reactions which occurs within the organs, and a replace organ's primary intended purpose would be to achieve these actions more effectively that the original organ it replaces [8]. Clearly 3D bioprinted organs fall outside the scope of this very definition. Although many researchers and clinicians along with industries hope FDA would classify printed organs as device by revising the regulatory terms but as of now the statutory language and analogies does not support this classification. There is exhaustive list of items that are treated as devices like metal implants but this clearly not same as “bio-ink” made from human biological materials and does not use cells in its manufacturing process. Furthermore, more often these implants are usually absorbable which is clearly not intention with the printed organs. Bioprinted organs are expected to function and be part of the body its implanted. Finally, closely analogies can be drawn as its replacement values derived from cadaver or animal

tissue; however, these organs or tissue are undergoing "minimal manipulation" before transplantation and intended to be used as "*replacement of diseased, damaged, malformed or malfunctioning native or prosthetics"*. This is exactly what 3D bioprinted organs intend to achieve; however, the critical difference is these values falls under Humanitarian Device Exemption (HDE) which was created as path for products that can be used in "diseases or condition that affect small (rare) populations." Rare disease is defined as condition that affects less than 8000 individual per year and bioprinted organs clearly does not qualify for this exception.

Finally, even the products and device that are like the products that FDA classifies as "device" are different from 3D printed organs in very important and critical ways. Additionally, the language itself no matter how similar it sounds to be describing bioprinted organs, is mismatch when describing its "primary intended purpose through chemical reaction inside the body" because the primary intended use of printed organ is to function as part of the body. The current regulatory terms and statutory language clearly does not mandate regulation of 3D bioprinted organs as medical devices [9].

There is need for scientist, clinicians and regulatory agency across the board to come up with guidelines that does not stifle the growth of 3D printing as this technology could help patient who otherwise must wait decades before finding matching donor, some of these patients do not even make it through the line. 3D printing hold great promises but as with great power comes greater responsibility and it falls on all the players involved in this technology to maintain and monitor social-ethnical boundaries.

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Conclusions

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Not many are aware of the fact that 3D printing has existed since late 1980s; with Charles "Chuck" Hull inventing sterolithography in 1984. Main reason not many people heard about the technology was because it was shuttered behind patents. Once the patent expired, 3D consumer was born. Biggest technological leap happened with engineering of BIOINKs that makes it possible to replace defected organs. 3D printer scans, designs and prints different organs and tissue with all its intrinsic details which is not possible by any other technology so far. There are many applications for 3D printing in industries including arts and fashion, medicine, architecture, automotive, and aerospace.

The 3D prints at the Prado Museum: in 2015, the most famous museum in Spain, organized an exhibition that featured paintings by Greco, Gentileschi and also Jose de Ribera, all of whom used 3D printer. This allowed the visually impaired people to feel these work, which was impossible previously. Entire work was created by start-up Estudios Durero. This is one of the examples showing impact of 3D printer and its ability to change how arts are conceived and visualized.

The biggest impact this technology has had is medicine. The medical professional discovered a technique with capability to engineer customized, organic forms with a degree of precision that has been impossible to achieve so far. Currently, bioprinting has been successfully used to manufacture human tissue like skin or cartilage. However, in near future more complex structures like heart and lungs will likely be possible. There are many research groups who are working exhaustively to engineer functional complex human organs that can improve living standards for patient suffering from chronic diseases.

3D printing has had considerable impact on drug industry. Researchers have designed and distributed compact rigs, which has ability to manufacture a single type of drug; they are by creating distributed production scenario. It technically means "3D printed chemical reaction vessels" that are called "reactionware" capable for making the drugs when reaction is initiated. Major advantage of this technique is it would prevent counterfeits and checks in place like each reactionware would be able to make the drug it is especially designed to make stops misusing of rig. Furthermore, this technique is also cost saving since mass-production of less often used drug does not result in profits for pharmaceutical companies and usually face supply shortage. This reactionware would address this situation, as the apparatus would produce drug-on-demand enabling wider distribution that in turn results in more hospital and patient equipped with drug they require.

These are only few examples of how 3D printer is game changer for many industries not just in medical arena. As 3D printing becomes more distributed and potent, new innovation opportunities will arise.

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Dr. Daniel J. Thomas BEng (Hons) MSc EngD CEnv CSci CEng FBCS.

Daniel J. Thomas is an interdisciplinary researcher and writer who comes from an academic background in 3D Printing, Engineering and Computational Design. This book '3D Printing in Medicine and Surgery' focuses on his current research as well as the research of numerous researchers who are engaged in developing new 3D Printing technologies that are currently treating patients and will lead towards the development of future treatments.

Dr. Deepti Singh MSc PhD.

Deepti Singh is a bioengneer who has been exploring the application of polymeric materials in restoring or repairing injured tissues and organs. With a Ph.D in genetics and biotechnology, she has worked extensively for the last decade to develop functional biomimetic that can enhance differentiation of embryonic stem cells into desired linage. This book focuses on interesting and new aspects of biomaterial design and its applications. With the emergence of bio-ink, 3D printing could potentially change the entire approach of regenerative medicine in which, instead of restoring and repairing, the disease tissue can be replaced.

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