Gels Horizons: From Science to Smart Materials

Anuj Kumar
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Vijay Kumar Thakur *Editors*

3D printable Gel-inks for Tissue Engineering

Chemistry, Processing, and Applications

Springer

Gels Horizons: From Science to Smart Materials

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Preface

Over the past decade, additive manufacturing technologies have emerged as one of the most wonderful fabrication methods in the field of tissue engineering and regenerative medicine due to their precise control and 3D printing of architectures as per tissue-specific or organ-specific demands using digital models based on medical imaging data. Among them, extrusion-based three-dimensional (3D) printing/bioprinting is a commonly used technique in tissue engineering areas. Various gel-inks or bio-inks have been developed and utilized for 3D printing by including natural and synthetic polymers, bioceramics, bioactive glasses, and glass-ceramics. Significant research has been performed in this area, but several challenges are remaining to be overcome in terms of data imaging, additive manufacturing technique, and design of biomaterial-inks (i.e. gel-inks or bio-inks) with their good printability and post-printing shape fidelity, 3D printing of curved and complex architectures, etc. Therefore, it is very important to understand the significance of 3D printing techniques, especially in tissue engineering applications, where healthcare issues are major concerns for human beings. A concise understanding and an overview of the chemistry and processing of biomaterials and 3D printing methods for various tissue engineering applications are provided in this book for the readers. Valuable knowledge is updated and organized according to various current studies worldwide in the field of tissue engineering.

This book provides an overview and discusses the chemistry, processing, and tissue engineering applications of the biomaterials that have been used for synthesizing 3D-printable gel-inks. This authoritative book provides the necessary fundamentals and background for researchers and research professionals who intend to work in the field of 3D bioprinting in tissue engineering. In 3D bioprinting, the design and development of the biomaterial-inks/bio-inks is a major challenge in providing a 3D microenvironment specifically to the anatomical and architectural demand of native tissues. Therefore, the main purpose of this book is to provide the basic chemistry of the biomaterials, their current processing developments and challenges, and recent advancements in tissue-specific 3D printing/bioprinting. The topics comprise mainly (1) biomaterial types, their synthesis and/or modifications, and processing for the particular 3D printing method, (2) characterization methods before printing and post-printing as well as *in vitro* and *in vivo* analyses, and (3) their applications and uses in various tissue engineering applications. This book serves as a go-to reference on bioprinting and is ideal for students, researchers, and professionals, including those in academia, government, the medical industry, and health care.

Chapter 1 provides the introduction to 3D printing technology for biomedical applications, describing the progress and development of printing technology to create organs or tissues including limitations. Chapter 2 presents the characterization of bio-inks for 3D printing applications by explaining numerous characterization methods. Chapter 3 discusses the 3D printing of hydrogel constructs toward targeted development in tissue engineering. Chapter 4 presents and discusses 3D-printable self-healing scaffolds for tissue engineering applications. Chapter 5 focuses on gel-inks for 3D printing in corneal tissue engineering applications. Chapter 6 introduces the current state of 3D-printable gel-inks utilized for skin wound treatments, whereas Chap. 7 presents biofunctional inks for 3D printing in skin tissue engineering applications. Chapter 8 explores the possibilities of using starch gels combined with different bioceramics for additive manufacturing and alternative fabrication methods for developing biomimetic implants for filling large bone defects, while Chap. 9 focuses on additive manufacturing of bioceramic scaffolds for bone tissue engineering applications by emphasizing stereolithographic processing. Chapter 10 is concerned with 3D-printable gel-inks for microbes and microbial structures to study microbes and microbe-host interactions, biofilm formation, antibiotic resistance, and microbiome through 3D modeling of microbes and infections for understanding diseases in a broader sense. Chapter 11 describes the methods of polysaccharide crosslinking, specifically future crosslinking methods of alginate hydrogels for 3D printing for biomedical applications, including tissue engineering areas. Lastly, Chap. 12 discusses the future perspectives for gel-inks for 3D printing in tissue engineering by considering precursors and other specific challenges.

At last, but most importantly, we would like to thank and acknowledge the authors who contributed to this book. In addition, we thank all reviewers for giving their valuable time to provide their reviews timely to improve the quality of this book.

Gyeongsan, South Korea — Anuj Kumar
Bucharest, Romania — Stefan Ioan Voicu
Dumfries, UK — Vijay Kumar Thakur

About This Book

1. Provides the background for 3D printing and tissue engineering and their challenges.
2. Provides the chemistry, functionalization, and processing of biomaterials.
3. Describes the pre-printing and post-printing processes for biomaterials according to particular 3D printing methods.
4. Discusses the efficacy of gel-inks for various tissue engineering applications.
5. Discusses the futuristic perspective in terms of 3D, 4D, and 5D printing/bioprinting technology.

Contents

About the Editors

Dr. Anuj Kumar is an Assistant Professor at the School of Chemical Engineering, Yeungnam University, South Korea. He has completed one research project (3 years) as Principal Investigator (PI) awarded by the National Research Foundation of Korea (NRF) and recently received two more sponsored research projects for 3 years one as a PI awarded by NRF (Korea) and one Indo-Korea joint research project as a Co-PI awarded by Korea Research Foundation (KRF, Korea) and Department of Science and Technology (DST, India). He worked as a Postdoctoral Research Associate in the Department of Nano, Medical and Polymer Materials (School of Chemical Engineering) at Yeungnam University, South Korea and Assistant Professor in the Department of Chemistry at DIT University, India. He received his Ph.D. in Polymer Science and Engineering (Polymers & Biomaterials) in 2014 from Indian Institute of Technology (IIT) Roorkee, India. He received his M.Tech. (Fibre Science and Technology) in 2009 from IIT Delhi, India and M.Sc. (Organic Chemistry) in 2006 from Chaudhary Charan Singh University, Meerut, India, respectively. He has published more than 55 SCI articles and 7 book chapters. He has rich experiences in the field of polymer/fibre science, lignocellulosic biomass, synthesis and processing of bio-based polymers, nanocelluloses, carbon nanomaterials, surface modification of polymers, polymeric composites/nanocomposites, chemistry and design of nanocomposite biomaterials, bioceramics and bioactive glasses, hybrid scaffolds and hydrogels, additive manufacturing (e.g., 3D printing/bioprinting), hybrid materials for cancer therapy, etc. His current research is focused on developing 3D printable hydrogels for various tissue engineering applications.

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Chapter 1
Introduction to 3D Printing Technology for Biomedical Applications

Satish Kumar, Ramaraju Bendi, and Vipin Kumar

Abstract The progress in tissue engineering and regenerative medicines has made organ replacement or regeneration easier, and its demand has increased rapidly in recent years. Bio-printing of human organs or tissues has become possible only because of the successful development of the bio-ink used in three-dimensional (3D) printing technology. Owing to the unique attributes of 3D printing, it can create an object of any complexity, including tissues with highly customized requirements for the subject (i.e., patient) specific applications. Development in smart materials, such as thermoplastics, powdered plastics, and photopolymers, enabled 3D printing to create objects with customized mechanical properties to mimic human organ models accurately. It brings new possibilities to create bionic tissues or organs, and it becomes even more desirable where the donor shortage is a severe problem. Despite cell printing, the effort remains to be made to accomplish the higher objectives of the in-vitro manufacturing of tissues or organs. This chapter sheds light on the progress and development of 3D printing technology to create organs or tissues. Also, the current state-of-the-art of the materials that can be processed, designed, is discussed comprehensively. The potential and major limitations of 3D printing technology in the field of bio-printing and related medical applications are discussed in brief.

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1 Introduction

With the revolution in medical technology, healthcare facilities have been increased seamlessly in recent years [1]. However, transplantation of organs or tissues required for lesions and defects has remained a crucial problem and is the subject of further investigations [2]. The existing techniques, such as auto-transplantation, xeno-transplantation, and artificial mechanical organ implantation found ineffective in improving the quality of transplant and the patient's life [3, 4]. For example, auto-transplantation, which exhibits satisfactory outcomes, but at the cost of antilogous-health-organization. It may cause various difficulties and inevitable side-effects [5]. Xeno-transplantation or heterologous transplant allows living cells, organs, and tissues to be transplanted from one species to another is readily used for end-stage organ failure [6]. This approach invites various potential challenges such as immunological rejection and viral transmission [7]. The implantable artificial organ in medical treatments is a quite successful approach and has significantly improved patient life [8]. The most developed artificial organs include the heart and kidney, while the pacemakers and cochlear implants are the most developed medical components [9, 10]. The implantable organs become mandatory when an organ in a person's body is damaged due to injury or disease. The crucial requirement of implantable artificial organs or prosthetics is to imitate the function of the original organ. Precise control over the physical and mechanical properties is essential for artificial biological organs [11]. Three-dimensional (3D) printing (3D printing) technology, which is known for its extreme controllability, is primarily employed in medical applications [12]. Owing to the unique attributes of 3D-printing technologies, which include high precession and speed, it is expected to overcome the crucial challenges encountered while using congenital methods/tools.

In addition to industrial, commercial applications, 3D printing technology, also known as the additive-manufacturing (AM) technique, is widely adopted by the medical industry [13]. This technique's working principle is based on the layered construction of the materials that are overlapped layer-by-layer [13]. In constructing an object with any complexity, the process involves well-optimized virtual-design objects using computer-aided designs (CAD). The optimization of CAD, appropriate selection of 3D printers and materials, plays a crucial role in producing a 3D object. These files serve as the guiding principle for the subsequent printing steps. The typical process steps are schematically illustrated in Fig. 1, which include the following steps: (a) CAD-assisted design of the object that contains the entire geometric information about the 3D objects; (b) steps-wise construction of 3D object through slicing the information into different 2D subsets; (c) periodic drying or curing of 2D subsets, (d) controlled movement of the stage along the z-direction, (e) repeat the steps (b) and (c) as per the printing duration. The process steps show that 3D printing involves the continuous addition of the materials on top of a previously cured or dry 2D layer. 3D printing technology opens a broad spectrum of vital opportunities for medical applications to create more specific human organs or tissues [14].

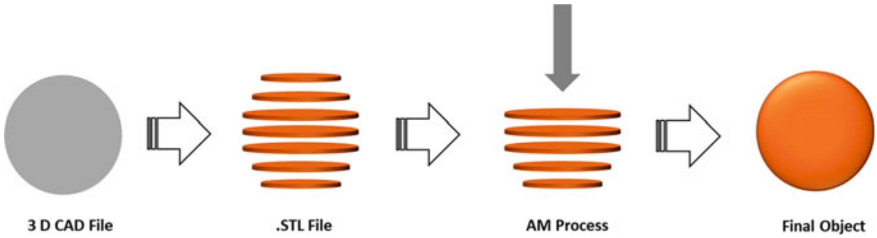

Fig. 1 Schematic illustration of the process steps involved during the development of a 3D printed object

Today, 3D printing technology has significantly developed the necessary research as well as skills for the new generation of surgeons [14]. Presently, the research on 3D printing technology for medical applications can be categorized into the following four primary areas of interest: (a) design and development of pathological organ models to help pre-operative planning and implant analysis [15], (b) personalized non-bioactive implants; (c) localized bio-active and bio-degradable supports or scaffolds, and (d) complete life-function of directly printed tissues or organs [16, 17]. Though the research focus remains far from the widespread medical applications due to various scientific and technical challenges, there are numerous printing techniques and materials available to give better results for tissue or organ designing. Nonetheless, some of the printing materials (i.e., printable biomaterials) are rigid and not suitable to meet the criteria of desirable flexibility and elasticity, progress in developing smart materials is made recently to fill the gap [18, 19].

2 Printing Mechanism: Classification of 3D Printing Techniques

Based on the printing mechanism, 3D printing can be classified into different categories. The commonly known printing mechanisms are given as follows; selective layer sintering (SLS), stereolithography (SLA), fused deposition modeling (FDM), and ink-jet printing. These mechanisms have their type of printable materials and advantages over others, see Table 1.

The strengths and limitations of each mechanism are described briefly in the following sections.

2.1 Selective Laser Sintering

This technique makes use of a high-power CO_2 laser, which is used to selectively fabricate the models in different steps. For example, 2D-slice data are generated

Table 1 Materials for different types of 3D printing techniques

Feature	SLS	SLA	FDM	Ink-jet
Materials	Metal (titanium, aluminide, stainless steel) and polymer powder	Polymer (light-sensitive) resin ceramic wax	Nylon, PLA, PVA, PC, wood-like, etc.,	Ceramic, plaster, plastic
Material's availability	Not-easily available	Easily available	Easily available	Easily available
Process	Chamber powder layer polymerization	Build plate basin exposure	Build plate extrusion layer bonding	Chamber powder layer bonding
Material cost	> $ 530/kg	> $ 700/kg	> $ 70/kg	> $ 350/kg

during the first step, which is put into the SLS machine that guides the laser's beam pathway. This laser beam scans the path on the powder surface, and this process heats it to sintering temperature, which makes it bond powder on the scanned path. After making the first fuse layer, the build layer descends, and the subsequent layer of powder can be put down and sintered. This procedure is repeated until the desired shape is accomplished. The un-fused powder on each layer serves as a supporting structure. During the sintering and cooling process, shrinkage and warpage become significant issues in the SLS method. These problems can be mitigated by using small-sized particle powder and airflow temperature within the sintering temperature window [20]. The advantage of this technique is that the product built by SLS can be reused even after being crushed into small pieces [21]. The schematics of SLS is shown in Fig. 2a.

2.2 *Stereolithography*

Stereolithography was originally known as the first 3D printing system and was invented in the 1980s. This technique's origin is based on photolithography, which is used to make a 2D pattern on the sample surface with high resolution. This technique is used to scan the UV light to cure the photo-resin with the desired pattern. After the UV exposure step, the resin tub moves up for a small distance in the z-direction to fill a new layer of resin on top or beneath the previous layer. The fill of the new layer by resin depends on the printer's configuration. Then, this newly filled resin layer will be again cured by UV light to make another pattern. This process repeats again and again until the complete 3D object is printed. The cost of this printer has reduced significantly because of the expiration of its significant patents in 2012. The high printing resolution in all x,y,z directions is the most remarkable advantage of the stereolithography technique. The shortcomings include lack of multi-material printing capability, small building dimensions, and it only works for photosensitive materials [22]. The schematics of the stereolithography is shown in Fig. 2b.

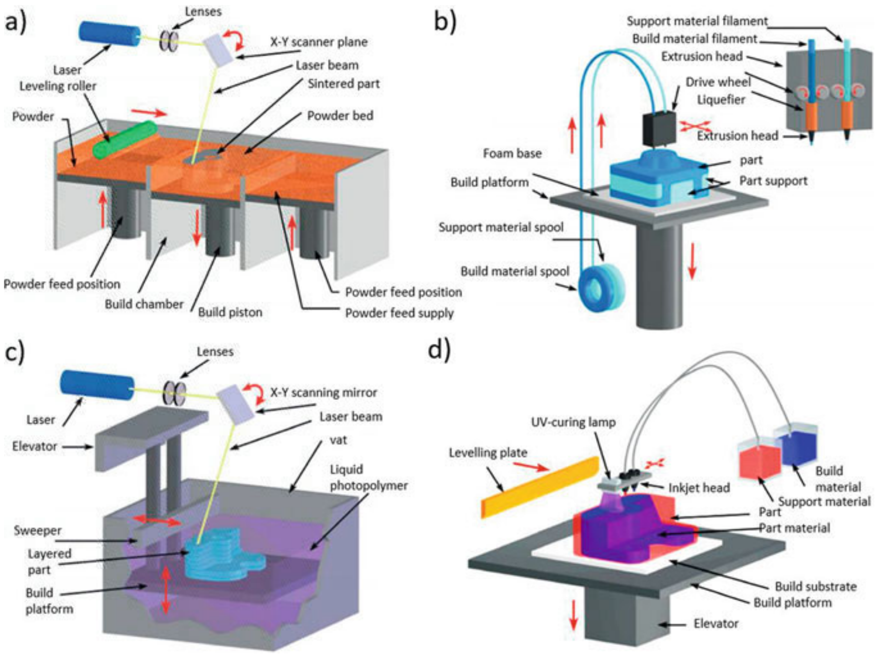

Fig. 2 Schematic illustration of the typical 3D printing mechanisms. **a** Selective layer sintering, **b** Stereolithography, **c** Fused deposition modeling (FDM), and **d** Ink-jet printing reproduced with permission [24]. Copyright @ 2018 Wiley–VCH Verlag GmbH & Co. KGaA, Weinheim

2.3 Fused Deposition Modeling

This method is another class of 3D printing mechanism in which the materials are extruded from the nozzle [23]. The jet is associated with a *x–y* driving system. The *x–y* motor system drives the nozzle to draw the pattern according to the layer information generated by the slicing software of the 3D printer. After finishing the pattern formation for one layer, the stage goes down to a certain distance, and the printer starts to follow the next layer's similar process. This is the most popular method for 3D printing. The expiration of patents made the printers affordable for the general public, and it is similar to stereolithography. This technique has better resolution (~300 μm) than that of the stereolithography, large building volume, low cost (less than 1 k USD), and multi-material printing capabilities that make it attractive for public and R & D activities. Nowadays, this method is widely used for bio-printing (3D printing biomaterial with or without cells). The schematics of the fused deposition machine is shown in Fig. 2c.

2.4 *Ink-Jet Printing*

The ink-jet 3D printing technique is similar to a desktop 2D ink-jet printer. Both methods have an array of the nozzle that is used to dispense tiny droplets of ink onto the substrate surface. In the next step, UV exposure is used to scan the entire layer to cure the droplets. After printing the first layer, a similar mechanism is used for another layer. The stage goes down to some distance, and the printing process repeats until finishing the whole object. This technique's resolution can be controlled by the droplet size, which is higher than that of the FDM printer; however, it is worse than the stereolithographic printer. The multi-material deposition is also feasible by dispensing different droplet types at the desired locations similar to that of the 2D color ink-jet printer. Typically, such printers are much more expensive (over 30 k USD) compared with the other contemporaries. The schematics of ink-jet printing machine is shown in Fig. 3d.

3 Evolution of 3D-Printed Medical Objects—Then and Now

Among 3D printing medical objects, bio-printing is the most recent and one of the attractive methods compared with many other technological developments. In the beginning, this research area was made extraordinary advances in some of the fields, and remained relatively stationary in others. There were limitations and technical

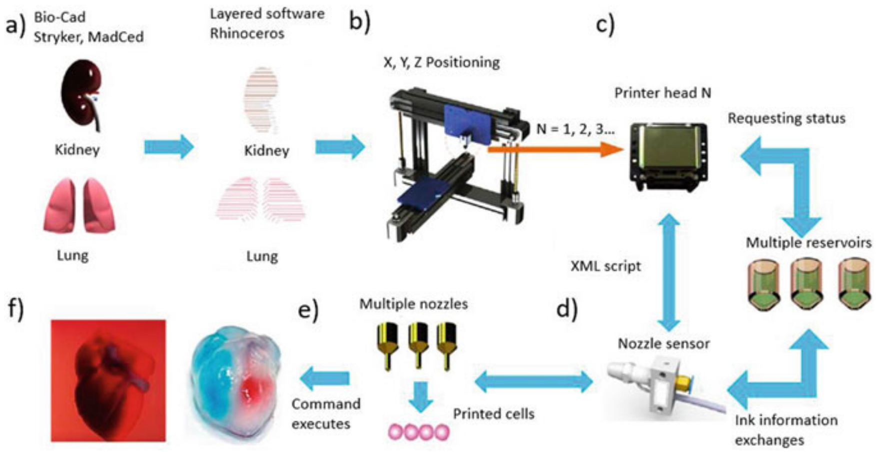

Fig. 3 Evolution and examples of bio-printing platforms. **a-e** Typical steps involved in the development of a human organ 3D-printed model, reproduced with permission [38]. Copyright @ 1969 Elsevier, **f** 3D-printed cardioid structure, reproduced with permission [37]. Copyright @ 2018 Wiley–VCH Verlag GmbH & Co. KGaA, Weinheim

challenges that are required to overcome before scaling-up this technique to the manufacturing level.

3D bio-printing moves up from the multidisciplinary combination of several other relatively cutting-edge technologies, for example, additive manufacturing, and cell patterning. Additive manufacturing technology is used for many other applications such as fabricating devices, components, and parts from materials such as metals and plastics. However, cell patterning and substrate patterning were related technologies used in R&D laboratories for probing cell–protein interactions. These techniques have been tried in combination to build a 3D living structure that could perhaps be used to replace the tissues and organs in a human patient [25–27]. The coined term was known as 'organ printing,' the precursor to 'bio-printing,' which we have accepted widely.

Bio-printing hardware consists of various parts and is shown in Fig. 3. Early bio-printers were based on custom-built, hacked, and ink-jet printers [29]. There are very few labs that are working in these areas to build their hardware. However, these custom devices were often incredibly difficult to operate because of full of software bugs and featured impossible user interfaces. Those lucky people who receive sufficient funding could utilize other 3D printing devices that were commercially available. Therefore, these devices were not engineered to print biological materials. The high cost for a single operational piece of hardware is around $ 100–200 k [30].

Meanwhile, the additive manufacturing technology continued to advance specifically in the open-source world, resulting in inexpensive but still buggy. Those printers were responsible for bio-printing only after substantial tinkering. Because of these limitations, bio-printing technology becomes more difficult but not impossible.

With time, the technology continued to evolve and bifurcate, as the terminology associated with other bio-printing facets. This progress was related to tissue liquidity and tissue fusion and developed a platform in which cell aggregates or tissue spheroids were deposited into a hydrogel biomaterial substrate. Both cell–cell and cell matrix-based interactions would fuse in a controllable manner and construct into a more extensive bioengineered tissue [29–38]. The aggregation of the cell was termed as bio-ink and bio-paper related to the hydrogel biomaterial component. However, bio-ink encompasses cells, biomaterials, and a combination thereof. More recently, a fully personalized, non-supplemented materials as bio-inks are demonstrated for printing of human organs (i.e., heart) [37]. The bio-inks consist of a fatty tissue, which is taken out from a patient and the bio-inks were processed from cellular and a-cellular materials. The potential of 3D-bio-printing technology to engineer vascularized cardiac patches is highlighted.

4 3D Printable Materials for Medical Applications

The specific materials that are used in 3D printing are allowed to transform to abide by the limitation of a specific model. The process can be executed in different steps; (i) materials' distortion by melting of a stiff filament; (ii) solidification of the melt in the desired form or construction of the structure; and (iii) solidification of the power. To allow these processes to occur, a filler or supporting or additive material is required, which is often accommodated in the lattice forms to mitigate distortion of the model during the curing step. These supporting materials or fillers can easily be removed or disintegrate from the parent structure by simply using hands or with a specified cutting tool. However, risk of leaving an impression on the surface is always there, and therefore it requires an additional polishing step to get-rid-of the marks. Though polishing is essential to obtain a good quality printing, this step may increase the risk of deformation of the structure, may lose fine details, and breaking of the geometry [39].

The selection of the material depends upon printing technique, printer technical specifications, and requirements of the model. The mechanical/elastic properties of the materials are chosen based on the structure of interest, for example, the anatomical structures are highly sensitive toward the mechanical/elastic attributes of the printed materials [40]. The key distinction between different materials that characterize the human body includes a combination of rigid tissues and soft materials. For instance, bones and ligaments (i.e., articular cartilage) represent rigid tissues and soft materials, respectively. The bones are the easiest and simplest biological tissue that is ever been produced by 3D printing technique with the majority of rigid materials. There are examples in 3D printing to model bone construction, such as acrylonitrile butadiene styrene (ABS) [39], powder of plaster [41], and hydroquinone [40].

However, 3D printing of the soft tissues is in infancy, and further development is the need of the hour to harness the full potential of the techniques. There is a need to conduct a depth research to fill the vacuum among a 3D-printed anatomical model and a true structure of human organ or tissue. Most of the 3D printing materials severely lack realism to mimic or duplicate a soft biological tissue, and an additional step, i.e., post-processing is necessary to soften the printed structures. There are some examples of the reproduction of cartilaginous tissues [42], arteries of practicing valve replacement [43], hepatic segment [44], and hearts [45]. Besides that, there is an interesting example in the development of 3D-printed brain aneurysm using a flexible TangoPlus™ photopolymer, which is a useful tool for the treatment of congenital heart disease [46]. There are several 3D printing processes that work on different operating principles and significantly vary in terms of technologies and materials' selection and niche area of applications. Also, it is worth mentioning that 3D printing seamlessly allows the development of the reproduction of implantable custom devices. However, there is a long way to go to adopt 3D-printed critical organs (e.g., heart) for implantations, and more profound research is still needed to examine the difference between traditional and additive manufacturing in mechanical and structural properties [47].

5 Significance of 3D-Printed Objects in the Medical Field

In many areas of medical field, 3D printing technology is indispensable in modern medical technology. Every year, this technology offers many healthcare field applications that help to save and improve our lives. Indeed, 3D printing has a wide range of applications in the field of healthcare, for example, cardiology [43], cardiothoracic surgery [47], gastroenterology [48], neurosurgery [49], oral and maxillofacial surgery [50], ophthalmology [51], otolaryngology [52], plastic surgery [53], podiatry [54], pulmonology [55], radiation oncology [56], transplant surgery [57], urology [58], and vascular surgery [59]. 3D printing technology deserves to be recognized at large scale in the field of healthcare and medical due to its ease of availability and operation. The leading direct applications of 3D printing in the medical and clinical fields are discussed comprehensively in the following sections.

6 Applications of 3D Printing

3D printing has retained a great passion and invention to the modern medical science. It is now promising and virtually effortless to offer modified health care solutions to help to medicinal practitioners and patients alike. It is projected that 3D printing technology will be worth over $3.5bn by 2025 in the medical field, compared with $713.3 m in 2016. The industry's compound annual growth rate is supposed to reach around 17.7% between the years 2017 and 2025. As one might witness the journey of 3D printing technology has enabled customization in medicine, prototyping, manufacturing, and academic research activities. 3D printing has many functions in medical sciences, for instance, this technology could be successfully applied to transplantation of human organs, expediting surgical process, low-cost production of surgical tools for surgery process, and may significantly improve the lives of those reliant on prosthetic limbs. As one might expect, the area of applications is quite broad, which extends from surgical to dental to implant tissue regeneration. The following are the specific application areas;

- Printing of surgical preparation
- Custom-made prosthetics
- Dental
- 3D printing of tissues, organoids, and tissue regeneration
- Medication dosage and pharmacology
- Manufacturing of surgical tools and medical metal materials

6.1 3D Printing of Surgical Preparation

In the human body, the individual variances and complexities are significantly great and 3D-printed models could be ideal for surgical preparation, as the printed tools can be customized to a great extent. For controlled and precise model development, the imaging techniques are essential for 3D printing technology. Besides surgical, 3D printing is equally revolutionizing medicine. 3D printing is being used to mimic patient-specific organs that are used for practice purposes to fix well before the actual complicated operations take place. The application becomes much better and more accurate if examines the results with X-rays, CT scans, and MRIs. Ultimately, this technique of surgical preparation has proven to pick up speedy procedures and minimizes the possibility of patient injury. Dissections often compromised with the proper pathology, so they offer an additional lesson in anatomy than a surgical patient's representation. Across the globe, research organizations, healthcare professionals, and hospitals are using 3D-printed anatomical frameworks as reference tools for pre-operative planning, intraoperative visualization, and sizing or pre-fitting medical equipment for both highly complex and routine procedures.

By using 3D-printed technology, we can produce sterile surgical instruments such as forceps, hemostats, scalpel handles, and clamps. 3D printing techniques not only produce sterile tools but some are also can be made very small and precise origami with ancient Japanese technology for practice. These tools can be used to work on microscopic areas without causing extra harm to the patient. The key advantage of using 3D printing over the traditional manufacturing methods is the low cost and speedy nature of the process to produce surgical tools.

3D-printed models could be incredibly useful to neurosurgeons by idiomatic expressions of the complex structures of the organs in the human body (Fig. 4). The radiographic 2D images are sometimes difficult to concealed right connections among cranial nerves, cerebral structures, vessels, and skull construction. Even a small mistake in navigating this complex anatomy can have potentially devastating consequences to both patient and the operating personnel. A realistic 3D model of skull helps better to speculate or predict the relationship between a scratch and typical brain structures. It can also help determine the protected surgical corridor and could be equally important for the neurosurgeon to practice critical operations. For example, 3D-printed models have recently been used to study complicated spinal deformities.

3D-printed models have been utilized in numerous situations to gain a deeper understanding of patient's specific anatomy before conducting an actual operation. Biotexture Wet Model [60] was developed by a Japanese company Fasotec, bought by Stratasys, to realistically mimicking real organs, for instance lungs, which are used to practice by both the surgeons and students prior to perform the actual operation in the operation theater. Nowadays, 3D-printed models are widely helpful for planning complicated surgery procedures, and commercially available in the market and common places. It has assisted full face transplants [61], the first adult-to-child kidney transplant [62], removal of a kidney [63], or liver tumor [64] in hospital and acetabular reconstructive surgery [65].

Japan's Kobe University Hospital, pioneering surgeons planned to liver transplantations with 3D-printed models. They used 3D-printed models of a patient's organs to understand the best possible ways to carve a donor's liver with negligible tissue damage to fit perfectly to the receiver's abdominal crater. For such applications, 3D-printed models are required to be partially transparent and prepared with very low-cost materials, for example, acrylic-resin or polyvinyl-alcohol (PVA) with excess water content. The texture of these models mimics living tissues, which gives an advantage to the surgeons, and allowing them to experience a live penetration by the surgical knife edges [66].

3D-printed model does not cost more than a custom made models by other techniques, however, the processing time is way lesser than the previously reported techniques [67]. Recently, a 3D-printed polypeptide chain model was developed and allowed to wrinkle into subordinate structures until it reaches a limit of bond rotation barrier and degrees of freedom [23]. Such models could be useful to gain insight into the other similar types of biological or biochemical structures (see Fig. 5). Several studies have been conducted on such origami structures and identified that the students could conceptualize the molecular structures better when demonstrate with the help of such models.

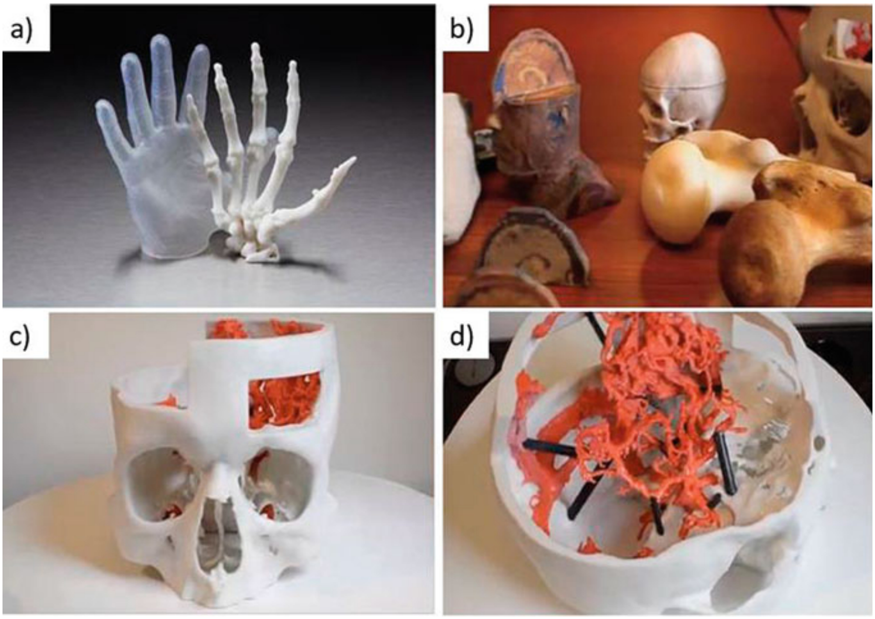

Fig. 4 **a.** Anatomical model of a hand, including the 'skin' made out of an elastic 3D printing material. **b.** Researchers at the National Library of Medicine generate digital files from clinical data, such as CT scans, that are used to make custom 3D-printed surgical and medical models. **c, d.** 3D model used for surgical planning by neurosurgeons at the Walter Reed National Military Medical Center. *Source* The Guardian (NIH 3D Printing Exchange)

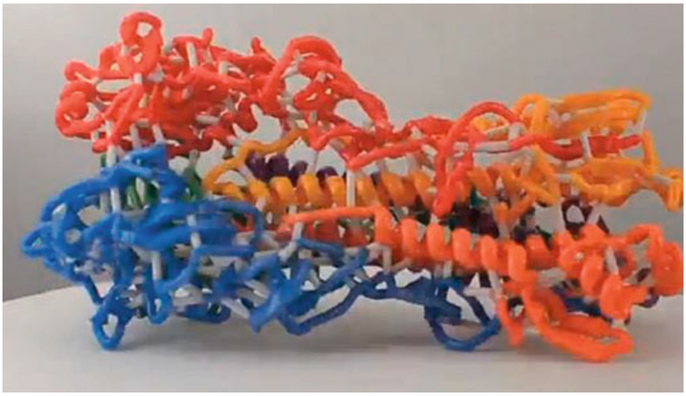

Fig. 5 An influenza hemagglutinin trimer 3D-printed model. *Source* The Guardian (NIH 3D Printing Exchange)

6.2 Custom-Made Prosthetics

3D printing has widely been used in the field of healthcare to model both normal and made-to-order prosthetic medical objects such as limbs and surgical implants [68–70]. This methodology has been used to construct dental, backbone, and hip transplants [68]. Earlier, surgeons had to execute bone-implant operations or use scalpels and drills to adjust implants by shaving pieces of metal and plastic to the anticipated shape/form, size, and fit, which is very time-consuming, and might not sound safe from patient's perspective. On the other hand, 3D printing has the ability to produce custom implants quickly. Prostheses solve an evident and persistent problem in orthopedics, where standard implants are often not sufficient/compatible with some patients, particularly in complex cases. A similar explanation is equally valid in neurosurgery. Due to irregular shapes and sizes of the skulls, it is very immensely challenging to regulate a cranial implant. The sufferers of skull wound, where the bone is discharged to give room to swell brain, the cranial plate that is later tailored must be unconditionally seamless. Although some of them are milled, more and more are developed using state-of-the art 3D printers, making it much stress-free to tailor, apt, and re-design, if required [70]. There have been many other viable and scientific accomplishments in the field of 3D printing of prostheses and transplants [23, 66, 68]. Researchers at BIOMED Research Institute in Belgium precisely implanted mandibular prosthesis of titanium (Ti) through 3D-printing technique [66]. The transplant was through a high-power laser to melt down thin layers of Ti-powder and developed the framework successively.

3D printing had a transformative effect on hearing aid production also. Today, 99% of hearing aids that suitably fit into the ear are custom-made by using any of 3D printing technologies. Each person's ear canal is shaped differently from others, and the use of 3D printing technology allows custom-shaped devices to be produced efficiently with low cost and no time [68]. 3D-printed modified hearing-aids to the market were facilitated because Class I medical devices for external use are subject to fewer regulatory restrictions. Envisaging braces are another successful commercial use of 3D printing, with 50,000 printed every day [70]. These clear, removable, 3D-printed orthodontic braces are custom-made and unique to each user. This product provides an excellent example of how 3D printing can efficiently and profitably make single, customized, intricate items at a relatively low cost and less time [70].

3D printing technologies have made researchers and industries to make highly customized prosthetic design and produce limbs at incredibly more affordable price for those who are lacking money and direct reach to the big cities. At present in the market prosthetics cost you a huge chunk of money. In the US market, it may cost a family anywhere from 5000 to 50000 dollars, causing a significant financial burden. Moreover, prosthetics need to be custom fit to the individual requirements, which demands additional production time of a few weeks to a few months. The ease of availability and operation of 3D printers offers the ability to a person to design and print customized parts, all of which have made prosthetics radically more affordable and accessible to the required people around the globe. Manmade hands and arms are some of the most commonly accepted 3D-printed prosthetics. Ivan Owen was designed a bionic hand in 2011 and he shared his experience and made the files open for others to print and distribute the same. His efforts and experience led to creating the e-NABLE Community through volunteers from a global network of 3D printing to develop in printing and designing prosthetic hands. These hands tend to cost only $50 compared with thousands of dollars which these individuals would have to pay. More technically challenging, Limitless Solutions has begun clinical trials for their 3D-printed prosthetic arms. These arms use muscle-flexing in the remaining portion of the arm, detected by leads attached to the skin, to guide the prosthetic movement. These arms are cost around $1000, which is a tenth of the typical $10,000 price point [71] (see Fig. 6).

6.3 Dental

The first few engineering applications of 3D printing were in the arenas of quick-tooling and instant-prototyping (see Fig. 7). Thus, its use in dentistry, where particular, custom-made objects were mass-produced, was an obvious next step in the flight of 3D printing technology. 3D printing applications in dentistry have helped in various ways, from orthodontics to general dentistry. Various dental areas integrating 3D printing technology are producing customized and accurate braces, castable crowns, dental restorations, dental bridges, denture frameworks, and bases. 3D printing technologies in the field of medicine had helped the dental health field to offer convenient chair-side care with a cheaper controlled treatment plan. It also reduces the waiting time due to avoiding molding step and the feature with high resolution can be printed directly. Another important aspect of 3D printing in dentistry is its easy adoption into

Fig. 6 14-year-old Sudanese Daniel Omar is fitted with a 3D-printed prosthetic. *Source* The Guardian [71]

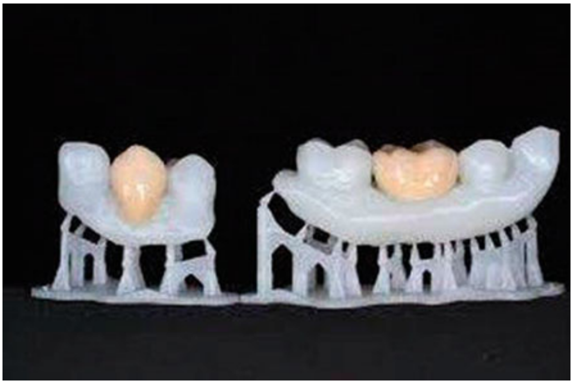

Fig. 7 3D-printed teeth in a reference model. (*Source* https://dental.formlabs.com)

a clinic, laboratory, or dental office. Speedy and accurately designed and developed solutions are critical to this application, and 3D printing in medicine does just what it requires.

Nowadays, by connecting oral-scanning, CAM/CAD design, and printing procedure, dental labs can rapidly and accurately yield various appliances, like pinnacles, connections, bandage/pebble models. In addition to that variety of orthodontic utilizations for instance, medical guides and aligners have been developed. In the place of painful imitations, a 3D scan is taken instead, later this 3D scan converted into a 3D prototypical and sent to a 3D printer to get the final print. 3D-printed prototypical can be used to generate a variety of orthodontic applications, distribution and positioning salvers, clear aligners, and retainers. Interestingly, the printed models can be conveniently stored digitally in the form of CAD (computer-assisted design) files.

This technique allows one to digitize the entire workflow, radically accelerating the construction time and considerably increasing the manufacturing capacity. Besides that, it allows one to eradicate the necessity of physical impressions and storing of replicas.

Today, there are various 3D printers are accessible in the market for both research and commercial purposes of dental and orthodontics. Besides 3D printing giant Stratasys (i.e., Stratasys for the 3D world [72]), there are various smaller companies or ventures available in the market such as Zenith 3D Printing Systems [73], Envision [74], AXSYS, Valplast, and many more. It is worth mentioning that the first FDA-certified 3D-printing material [75] and a new material (i.e., bio-ink) that kills bacteria [77] have been developed in recent years. 3D printed surgical-guides find their potential applications in dental practice, but remained unnoticed [79]. To highlight numerous low-cost printer for small, medium, and more extensive dental labs and clinics with unique accessible operation have been developed for both demonstration and serious medical facilities. The new materials that secure complete applications of 3D printing in dentistry need to be developed for constant growth of the field and applications. These 3D printers are in trend across the USA and will undoubtedly be followed by rest of the countries around the globe. Interestingly, the first, to the best of our knowledge, liability case concerning 3D printing (of dental aligners) has been submitted to a court in California [80].

6.4 3D Printing of Tissues, Organoids, and Tissue Regeneration

Manufacturing humanoid tissue by 3D printing is an exciting yet relatively untapped, flourishing area of potential applications [81–84]. 3D bio-printing envisages to reduce the shortage of supply of the critical organs in the donor market. This is incredibly applicable and exciting to the field of transplantation, as it solves any ethical and moral issues that may be tied to traditional transplant methods. It also increases acceptance as customized organ development utilizes the patient's cells. Skin tissue reconstruction and repair, kidney transplantation, heart transplantation, and limb replacement among others are being successfully attained through 3D printing technology in recent years. Bone and muscle repairs also have been possible with 3D printing in medicine with orthopedic implants. Being able to 3D print tissue cells and organs has promoted research work for diseases, like cancer to study how tumors grow and develop, with the intent to find a cure.

The critical application of lab-on-a-chip, i.e., exVive3D Liver, is a high-yield 3D-bioprinted tissue prototypical tool for scientific exploration, medicine finding, and toxicology [85], which has proposed in recent years. In recent times, the possibility of bio-printing using stem cells has unlocked new possibilities in this realm [84]. The very-first bio-ink [86] is offered by the Swedish startup company CELL-Ink [87] and the American stem-cell company Rooster Bio [88]. Reasonable and consistent ingredients such as cell-friendly biomaterials are the fundamental requirements for a structure to be 3D printed. Uniform tissues, for instance, skin cells (printing skin cells on burn wounds), muffled and intricate solid organs, for example, a liver [90], have been printed through 3D printers using suitable bio-materials. Researchers from Chinese academy reported 3D bio-printing of the kidney (How do they 3D print kidney in China [91]), ears, and livers [92]. Although much progress is still required to make them implantation ready, preliminary studies on bio-printing of hard tissues such as bones (CT-Bone®: actual bone produced through 3D Printer [93]) and soft tissues such as cartilage and muscles [94] and other tissues have consistently been conducted to better understand their printing behaviors. Also, there is a novel thrilling substance, called self-healing bio-glass, that can be 3D printed and be used a cartilage replacement [95]. Recently, Atala and co-workers stated that the building of a combined tissue–organ printer (ITOP) can construct stable, human-scale tissue constructs of desired shape/size. The competencies of the ITOP are demonstrated by constructing jawbone and calvarial bone, cartilage, and skeletal muscle [96].

Plenty of 3D bio-printers introduced in the past decade, a low-cost bio-printer, by the Swedish startup CELL-Ink was displayed, which costs about 5000 $ for the less expensive model, and about 9000 $ for the more sophisticated version [97]. This printer allowed CELL-Ink to achieve remarkable outcomes, and it comprises of about 98% of alive cells when using their bio-ink in 3D printer. Not precisely a bio-printer but a tool called Bio-pen allowed surgeons to mending spoiled bone and cartilage by "sketch" new cells directly onto bone. This procedure could be conducted in the middle of a surgical process (see Fig. 8) (BioPen to redraft orthopedic implants surgery [98]). 3D-printed organs for replacements are still far away to grasp. Researchers from China, Xu Mingen, developer of the "Regenovo" bioprinter, projected that completely serviceable 3D-printed human organs are likely to develop in the time span of 20 years [99].

3D bio-printing has evolved as an effective tool to develop implants that accelerate bone regeneration under both *in-vitro* and *in-vivo* conditions [100–102]. The unmatchable attributes and technical capability of 3D printing create high resolution, repeat, and ordered porous scaffolds from a wide variety of materials (which include most of the metals, ceramics, and polymers). Studies have identified that a porous network could be an effective promoter of bone ingrowth. Through a traditional bone filling process, it is immensely difficult to manage critical-size bone defects, which, in most of the instances, leads to significant morbidity. A traditional bone filling method might not be effective to ensure a perfect bone integration, as a coherent blood supply to the graft is critical and essential, which is difficult to achieve through traditional filling methods. Though a coherent blood supply can be maintained under vascularization, this process requires a bone to be operated multiple times, which may lead to increase the possibility of morbidity.

Fig. 8 Carrying a special healing bio-ink, a Bio-pen being used on a bone model. (*Source* credit: University of Wollongong, Australia)

6.5 Medication Dosage and Pharmacology

3D printing in the field of drug delivery can possibly streamline pharmacology and drug administration. A modest explanation for patients with manifold disorders is made possible with a 3D-printed capsule that accommodates numerous drugs at once, each with a specific release time. An exemplary idea called ‘Polypill’ has been tested for diabetic patients. This application deals with the medication dosage and also solves issues of a diverse drug interaction. For the patient, it removes comprehensive 24-h care of medicine intake when their medications have different schedules. 3D-printed medicines in the Polypill concept can be very cost-effective. This makes the technology available to poor, developing countries and applicable to health programs at an affordable price.

Researchers at University College London have fabricated topical drug delivery systems using 3D bio-printing [103]. They investigated fused deposition modeling (FDM) and stereolithography (SLA) for the fabrication of devices to be worn on the nose and deliver salicylic acid for the treatment of acne. The salicylic acid is loaded into commercial polymer filaments using hot-melt extrusion. 3D printing lends itself to this process, as scanned images of the patient’s anatomy can be used to create devices that fit precisely, maximizing contact and delivering an even dose of the drug. They found that while both methods created suitable devices, the SLA method was more convenient as a fabrication process. The dosage can also be varied when the filaments used for printing are prepared.

To demonstrate 3D printing capacity to produce drug tablets of sufficient quality for prescriptions, Khaled et al. at the University of Nottingham attempted to print Guaifenesin Bilayer tablets (Mucinex) using a desktop 3D printer bought for under $1,000 [107]. They compared the drug release profiles for their designs and found

Fig. 9 Cube, pyramid, cylinder, sphere, and torus paracetamol tablets. Reproduced with permission [108].

that one of them showed a cumulative drug release profile that remained within 10% of the release profile of the commercial drug over a 14-h dosage cycle. They also evaluated the weight variation, hardness, thickness, and friability of the tablets they had produced. Given the new design freedom that 3D printing in pharmaceuticals provides, Goyanes et al. investigated the effect of tablets' different shapes on drug-release profiles [108]. They investigated torus, pyramid, cube, sphere, and cylinder shapes using an FDM process to print paracetamol-loaded filaments of PVA. Their printed tablets are shown in Fig. 9. They first demonstrated that the stability of the drug was unaffected by the printing process. They then investigated the amount of the drug that was released in each tablet and showed, as expected, a clear dependence on the surface area to volume ratio. They state that these complex geometries would be impossible to fabricate using traditional powder compaction methods and better control drug-release profiles.

A precise control over the amount of drug release is important for transdermal applications, as a high dose might perforate the skin tissues or may lead to skin infection. The natural distribution network of skin serves as a medium for the sustained release of a multitude of transdermal drugs molecules [104]. Presently, transdermal drugs are delivered through patches, which cover a large area of skin to enhance the effectiveness of the drug. A continuous drug delivery through patches can be provided to the upper layer of epidermis. On the other hand, a micro-needle array penetrates the upper layer of skin without affecting its integrity is effective and promising approach. In the context of transdermal drug delivery, 3D printing can offer an advantage in developing simple and complex patches or micro-needle arrays structures. The complex structure of patches or arrays is designed to allow drug release at different rates [105]. 3D printing techniques, for instance, SLA, have previously been employed to print micro-needle arrays with a high degree of precision with a wide variety of materials [106].