We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS

**BOOK
CITATION
INDEX**

INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Application of 3-D Printing for Tissue Regeneration in Oral and Maxillofacial Surgery: What is Upcoming?

Seied Omid Keyhan, Hamidreza Fallahi,
Alireza Jahangirnia,
Mohammad Taher Amirzade-Iranaq and
Mohammad Hosein Amirzade-Iranaq

Additional information is available at the end of the chapter

Abstract

The ultimate goal of any surgical procedure is to improve perioperative form and function and to minimize operative and postoperative morbidity. In recent years, many exciting and novel technological advances have been introduced in the field of oral and maxillofacial surgery. One example of such technology that is continuing to increase in prevalence is the use of 3-dimensional (3-D) printing techniques with special properties, which seems hopeful for practitioners in the field of regenerative medicine. Tissue engineering is a critical and important area in biomedical engineering for creating biological alternatives for grafts, implants, and prostheses. One of the main triad bases for tissue engineering is scaffolds, which play a great role for determining growth directions of stem cells in a 3-dimensional aspect. Mechanical strength of these scaffolds is critical as well as interconnected channels and controlled porosity or pores distribution. However, existing 3-D scaffolds proved less than ideal for actual clinical applications. In this chapter, we review the application and advancement of rapid prototyping (RP) techniques in the design and creation of synthetic scaffolds for use in tissue engineering. Also, we survey through new and novel merging era of "bioprinting."

Keywords: 3-D printing, prototyping, tissue engineering, scaffolds, bioprinting, stem cells, regenerative medicine, oral surgery, maxillofacial surgery

1. Introduction

Three-dimensional printing—also known as rapid prototyping—was first introduced in 1980s; during past three decades, enormous changes and development have been performed by scientists through modifying this technology by uses, material, and also accuracy.

With increasing attention of scientific societies, recently, scientific literature bolded feasibility of 3-D-printed tissues and organs and its usage within laborious clinical situations. Also, this technology was used largely in accurate and highly customized devices, such as tracheobronchial splints, bionic ears, and even more. Within the field of craniofacial surgery, 3-D surgical models have been used as templates to create bone grafts, tailoring bioprosthetic implants, plate bending, cutting guides for osteotomies, and intraoperative oral splints. Using 3-D models and guides has been shown to shorten operative time and potentially reduce the complications associated with prolonged operative times.

The goal of surgical procedures for a clinician is to improve perioperative form, recovery of function, and also minimizing operative and postoperative morbidity. Many exciting and new technological advances have ushered in a new era in the field of oral and maxillofacial surgery over the last years, which within no exaggeration 3-D printing is the novelist and controversial one.

The aim of this chapter is to introduce 3-D printing method and its role in the contemporary oral and maxillofacial surgery and to review current advantages of its application in the field of regenerative medicine.

1.1. History of the technology

Three-dimensional (3-D) printing has been utilized in diverse aspects of manufacturing to produce different objects from guns, boats, and food to models of unborn babies. From over 1450 articles related to 3-D printing listed in PubMed, nearly a third of them were solely published in the last 2 years [1].

Three-dimensional (3-D) printing is a manufacturing process that objects are fabricated in a layering method during fusing or depositing different materials such as plastic, metal, ceramics, powders, liquids, or even living cells to build a 3-D matter [2, 3]. It is a process of generating physical models from digital layouts [4, 5]. This technology demonstrates a technique that a product designed through a computer-aided scheme is manufactured in a layer-by-layer system [6]. This process is also cited as rapid prototyping (RP), solid freeform technology (SFF), or additive manufacturing (AM) [7].

3-D printing techniques are not brand new and have been existed since 30 years ago [8–10]. This technology is first introduced and invented by Charles Hull in 1986, and at first, it was utilized in the engineering and automobile industry for manufacturing polyurethane frameworks for different models, pieces, and instruments [11]. Originally, Hull employed the phrase "stereolithography" in his US Patent 4,575,330, termed "Apparatus for Production of Three—Dimensional Objects by Stereolithography" published in 1986. Stereolithography technique included subjoining layers over the top of each other, by curing photopolymers with UV lasers [12, 13].

Since then, 3-D models have been used for a diversity of different objectives. Since 1986, this process has started to accelerate and has honored recognition globally and has influenced different arenas, such as medicine.

The developing agora for 3-D desktop printers encourages wide-ranging experimentations in that subject. Generally, medical indications of these printers are such as treatment planning, prosthesis, implant fabrications, medical training, and other usages [4].

Having being used in military, food industry, and art, rapid prototyping is receiving a lot of attention in the field of surgery in the last 10 years [6, 14].

The pioneering usage of stereolithography in oral and maxillofacial surgery was by Brix and Lambrecht in 1985. Later this technique was used by them for treatment planning in craniofacial surgery [15].

In 1990, stereolithography was used by Mankovich et al. for treating patients having craniofacial deformities [16, 17]. They used it to simulate bony anatomy of the cranium using computed tomography with complete internal components [17, 18].

By aiding in complex craniofacial reconstructions, 3-D printing has recently earned reputation in medicine and surgical fields [19–21].

Today, maxillofacial surgery can benefit from additive manufacturing in various aspects and different clinical cases [22]. This technique can help with bending plates, manufacturing templates for bone grafts, tailoring implants, osteotomy guides, and intraoperative occlusal splints [23–27]. Rapid prototyping can shorten surgery duration and simplify pre and intraoperative decisions. It has enhanced efficacy and preciseness of surgeries [10].

2. Current 3-D printing techniques used in oral and maxillofacial surgery

From first innovation till nowadays, there are different kind of technologies introduced for 3-D printing. Binder jetting (BJ), electron beam melting (EBM), fused deposition modeling (FDM), indirect processes, laser melting (LM), laser sintering (LS), material jetting (MJ), photopolymer jetting (PJ), and stereolithography (SL) are well-known technologies of 3-D printing [14, 28, 29].

There are many different 3-D printing techniques. Benefits and disadvantages are factors to differ each technology system [14]. Among this variety of different techniques, there is a huge discussion and usage in oral and maxillofacial region for SL, FDM, and PJ [1, 28, 30].

Each technology has its own characteristics, properties, and advantages which **Table 1** summarizes some different three dimensional printing technologies.

3. Biomaterials available for 3-D printing

As researchers aim to investigate new materials for 3-D printing in last decade, it is obvious to see variety of biomaterials with different properties and also different applications. As **Table 2** summarizes all biomaterials used within studies all over the world for generating scaffolds for bone tissue engineering, it has to be noticed that from this large spectrum of biomaterials

Techniques	Advantages	Disadvantages
Light cured resin		
1. Stereolithography (SLA) Light sensitive polymer cured layer by layer by a scanning laser in a vat of liquid polymer.	Rapid fabrication. Able to create complex shapes with high feature resolution. Lower cost materials if used in bulk.	Only available with light curable liquid polymers. Support materials must be removed. Resin is messy and can cause skin sensitization and may irritate by contact and inhalation. Limited shelf life and vat life. Cannot be heat sterilized. High cost technology.
2. Photojet—light sensitive polymer is jetted onto a build platform from an inkjet type print head, and cured layer by layer on an incrementally descending platform.	Relatively fast. High resolution, high-quality finish possible. Multiple materials available various colors and physical properties including elastic materials. Lower cost technology.	Tenacious support material can be difficult to remove completely. Support material may cause skin irritation. Cannot be heat sterilized. High cost materials.
3. DLP (digital light processing) Liquid resin is cured layer by layer by a projector light source. The object is built upside down on an incrementally elevating platform.	Good accuracy, smooth surfaces, relatively fast. Lower cost technology.	Light curable liquid polymers and wax-like materials for casting. Support materials must be removed. Resin is messy and can cause skin sensitization, and may be irritant by contact Limited shelf life and vat life. Cannot be heat sterilized. Higher cost materials.
Powder binder		
Plaster or cementaceous material set by drops of (colored) water from 'inkjet' print head. Object built layer by layer in a powder bed, on an incrementally descending platform.	Lower cost materials and technology. Can print in color. Un-set material provides support Relatively fast process. Safe materials.	Low resolution. Messy powder. Low strength. Cannot be soaked or heat sterilized.
Sintered powder		
Selective laser sintering (SLS) for polymers. Object built layer by layer in powder bed. Heated build chamber raises temperature of material to just below melting point. Scanning laser then sinters powder layer by layer in a descending bed.	Range of polymeric materials including nylon, elastomers, and composites. Strong and accurate parts. Self-supported process. Polymeric materials—commonly nylon may be autoclaved. Printed object may have full mechanical functionality. Lower cost materials if used in large volume.	Significant infrastructure required, e.g., Compressed air, climate control. Messy powders. Lower cost in bulk. Inhalation risk. High cost technology. Rough surface.
Selective laser sintering (SLS)—for metals and metal alloys. Also described as selective laser melting (SLM) or direct metal laser sintering (DMLS). Scanning laser sinters metal powder layer by layer in a cold build chamber as the build platform descends. Support structure used to tether objects to build platform.	High strength objects can control porosity. Variety of materials including titanium, titanium alloys, cobalt chrome, stainless steel. Metal alloy may be recycled. Fine detail possible.	Elaborate infrastructure requirements. Extremely costly technology moderately costly materials. Dust and nanoparticle condensate may be hazardous to health. Explosive risk. Rough surface. Elaborate post-processing is required: Heat treatment to relieve internal stresses in printed objects. Hard to remove support materials. Relatively slow process.
Electron beam melting (EBM, Arcam). Heated build chamber. Powder sintered layer by layer by scanning electron beam on descending build platform.	High temperature process, so no support or heat treatment needed afterwards. High speed. Dense parts with controlled porosity.	Extremely costly technology moderately costly materials. Dust may be hazardous to health. Explosive risk. Rough surface. Less post-processing required. Lower resolution.

Techniques	Advantages	Disadvantages
Thermoplastic		
Fused deposition modeling (FDM) First 3-DP technology, most used in 'home' printers. Thermoplastic material extruded through nozzle onto build platform.	High porosity. Variable mechanical strength. Low- to mid-range cost materials and equipment. Low accuracy in low cost equipment. Some materials may be heat sterilized.	Low cost but limited materials—only thermoplastics. Limited shape complexity for biological materials. Support material must be removed.

Table 1. 3-D printing modalities and materials [14, 31].

Composed scaffolds	Synthetic scaffolds		Natural scaffolds	
Nano-hydroxyapatite/collagen/PLLA	Ceramic	Polymeric	Inorganic	Organic
Octacalcium phosphate/collagen	Calcium Magnesium Phosphate cement (CMPC)	PLGA	Silver	Collagen sponge
Nano-hydroxyapatite/polyamide 6	βTCP	PLG	Coral	PRP
Nano-hydroxyapatite/polyamide66	HA/TCP	PLLA	Silk fibroin protein	Gelatin sponge
Hydroxyapatite-coated PLGA	Flurohydroxyapatite	PGA	Premineralized silk fibroin protein	Gelatin Hydrogel
HA/PLGA	Ca deficient hydroxyapatite (CDHA)	PLA	ABB	PuraMatrix
βTCP/collagen		PLA-PEG	Deer horn	Alginate
DBM/PLA		Fibronectin-coated PLA		Partially demineralized bone matrix
Nano-hydroxyapatite/polyamide		PEG-DA		Bio-Oss
OsteoSet		PEG-MMP		Allograft
Octacalcium phosphate precipitated (OCP) alginate		PVDC		Fibrin sealant
Demineralized bone powders/PLA		Polycaprolactone		Gelatin foam
Apatite-coated PLGA				Collagen gel
				Hyaluronic acid based hydrogel

TCP, tri-calcium phosphate; HA, hydroxyapatite; DBM, demineralized bone matrix; PLGA, poly(lactic-co-glycolic acid); PLA, poly(D,L-lactic acid); PGA, poly(glycolic acid); PLLA, poly(L-lactic acid); PVDC, polyvinylidene chloride; PEG, polyethylene glycol; DA, diacrylate; MMP, matrix metalloproteinases; ABB, anorganic bovine bone; Puramatrix, a self-assembling peptide nanomaterial.

Table 2. Types of scaffolds used in bone tissue engineering in maxillo-craniofacial region [51].

just a whole bit of them are available for application in 3-D printing. As follows, we discuss four large categories of materials for 3-D printing of scaffolds and craniofacial tissues, which researches still aim to determine these materials complete properties and advantages.

3.1. Polymers and hydrogels

Polymer hydrogels are ideal candidates for the development of printable materials for tissue engineering. Hydrogels are known for remarkable tunability of rheological also presenting great mechanical, chemical, and biological properties; high biocompatibility; and similarity to native extracellular matrix (ECM) [32]. For three-dimensional printing of polymers and hydrogels, the use of materials with controlled viscosity should been noticed. This defines the range of printability of the ink. Polymer inks, which are typically printed in the prepolymer phase, need enough viscosity allowing structural support of subsequent printed layers, also enough fluidity to prevent nozzle clogging. For avoiding these difficulties, alginate hydrogels have been cross-linked with calcium ions immediately before the ink leaves the printing head or just after extrusions [33].

In recent researches, for providing suitable ink for bioprinting applications, prepolymerized cell-laden methacrylated gelatin hydrogels have been used successfully [34, 35]. Synthetic hydrogels used for cell encapsulation may limit cell-cell interactions. These interactions are critical for efficient cell proliferation, differentiation, and finally, tissue development. This can represent one of the limitations of bioprinting cell-laden hydrogels which is not present in 3-D printed scaffolds with cells seeded onto or in bioprinting of dense cell aggregates, which will discuss as follow. Hence, the requirement for the development of ECM-derived hydrogels that have tunable physical and chemical properties, are compatible with high cell viability, and provide the adequate binding sites (RGDs) for cell attachment and matrix remodeling during their early proliferative stage [32].

Synthetic polymers are most commonly used materials for 3-D printing in biomedical applications [36, 37]. However, since high temperature is usually involved during the printing of these materials, the direct incorporation of cells or growth factors in the polymer mixture is generally avoided as the cell viability or bioactivity [37] cannot be maintained throughout the manufacturing process.

Although hydrogels provide great advantages for tissue engineering applications, such as the ability of exposing cells to highly hydrated 3-D microenvironments that is similar to the natural ECM [32]. In contrast, they generally present very low stiffness (in the kPa range) compared with the majority of load-bearing tissues in the craniofacial complex (in the GPa range). Therefore, reconstruction of tissues subjected to higher mechanical loads, such as bones and teeth, usually requires the use of ceramic materials or composite scaffolds which provide more mechanical advantages, where polymers are commonly combined with inorganic fillers to increase scaffold stiffness [38].

3.2. Ceramics

Ceramic scaffolds are usually composed of calcium and phosphate mineral phases, such as hydroxyapatite [39] or b-tricalcium phosphate [40]. The noticeable ability of these scaffolds to upregulate osteogenesis due to inherent properties of the formation of a bioactive ion-rich cellular

microenvironment, also as mentioned before their ability to mechanically provide space maintenance, makes these materials interesting choice for 3-D scaffold fabrication for craniofacial applications. In contrast, ceramic scaffolds are not compatible with cell encapsulation for bioprinting. In 3-D printed ceramic scaffolds, cells quickly populate the scaffold surface, which establishing close cell-cell interactions lead to promotion of cell proliferation and differentiation. On the other hand, ceramics with properties lead to lower rates of degradation than hydrogels, which aids in prolonged guided tissue remodeling and structural support. In contrast, ceramic scaffolds are too brittle for implantation in load-bearing defect sites. Ideal scaffolds would combine the high calcium content of calcium and phosphate ceramics with the outstanding toughness of natural bone, which perhaps can only be obtained by creating scaffolds that are biomimetically mineralized and hierarchically structured, as recent researches demonstrated that in [41].

Fused deposition of ceramics (FDC) in a direct printing mode generally consists of extruding a slurry including a high content (>50% w/v) of inorganic components [42]. The manufacturing of such scaffolds follows 3 steps:

1. Mixture phase, which involves the preparation of the slurry. The bioceramic particles are mixed in a solvent (aqueous or nonaqueous) with a low concentration of organic polymers/surfactants, called the binder, to obtain adequate flowability.

2. Green ceramic and binder burnout phase involving the deposition of filaments of slurry following a predetermined pattern prior to drying and exposure to high temperature to burn out the organic component of the mixture.

3. Sintering phase, which involves the exposure of the green form to elevated temperature (above 1000°C) to initiate the migration of atoms between adjacent ceramic particles, hence creating physical bonds called "necks."

It is critical for reproducible manufacturing of 3-D rapid prototyped bioceramics to have shape retention, a challenge that can be reached by adjusting the viscosity of the slurry and the evaporation rate of the solvent [43].

3.3. Composite materials

Printable composites, which are usually in the form of copolymers, polymer-polymer mixtures, or polymer-ceramic mixtures [44], allow ability for the combination of variety of advantageous properties of their included components, which provide a remarkable candidate as "bioink". Considering the advantages of polymer composite hydrogels, such as interpenetrating polymer networks (IPNs) or hybrid hydrogels [45], the incorporation of synthetic fillers to printable materials recently discussed in researches [33]. The addition of silicate fillers [38] and a range of nanoparticles have been used to synthesize different types of composite scaffolds [46] to promote greater control over viscosity and stiffness of polymer hydrogels. In addition, several of silica-containing hydrogels with higher expression of genes encoding morphogenetic cytokines, such as bone morphogenetic proteins (BMPs) seems promising [47]. The combination and manufacturing mixture of hydrogels with filler materials and/or natural peptides with morphogenetic capacity demonstrate great future for application in 3-D printing in aim to reach ultimate goal in regenerative craniofacial repair.

3.4. Cell aggregates and spheroids

Over recent years, many of researches aimed to evaluate and study cell aggregates and spheroids for use in tissue engineering and regenerative medicine [48]. As this method cited correctly and appropriately as "scaffold-free printing," in fact small quantities of hydrogel are used to facilitate cell aggregation. In this method for 3-D printing, or in an appropriate
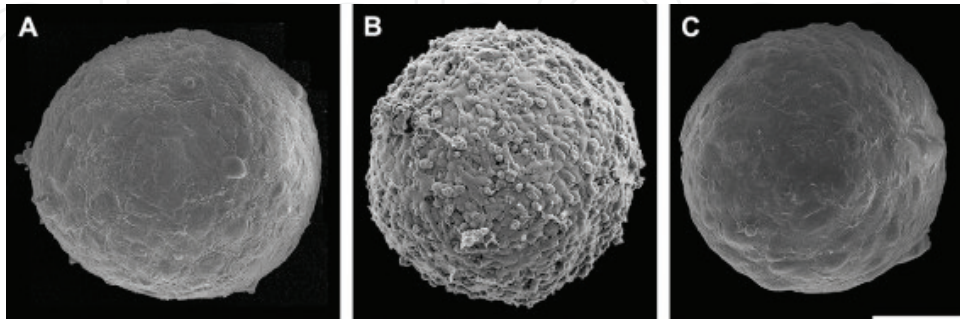

Figure 1. SEM view of multicellular spheroids of HUSMCs (A), CHO cells (B) and HFBs (C) (adapted from Norotte et al. [50]).
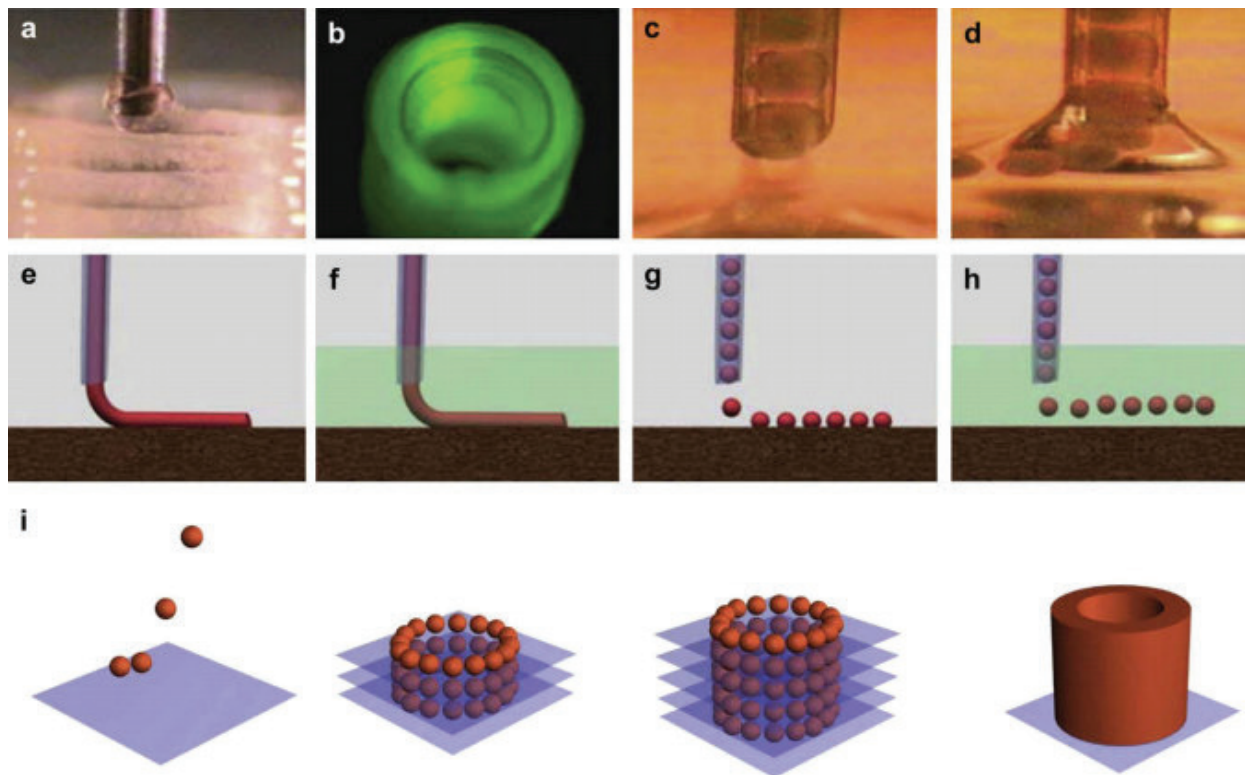
Figure 2. Principles of spheroids bioprinting technology: (a) bioprinter (general view); (b) multiple bioprinter nozzles; (c) tissue spheroids before dispensing; (d) tissue spheroids during dispensing; (e) schematic view of continuous dispensing in air; (f) schematic view of continuous dispensing in fluid; (g) schematic view of digital dispensing in air; (h) schematic view of digital dispensing in fluid; (i) schematic view of bioassembly of tubular tissue construct using bioprinting of self-assembled tissue spheroids illustrating sequential steps of layer-by-layer tissue spheroid deposition and tissue fusion process (adopted from Mironov et al. [48]).

way called "bioprinting," multicellular spheroids are deposited using extrusion printers and allowed to self-assemble into the desired 3-D structure (**Figure 1**). As it is clear, these systems allow direct fabrication of tissue constructs which in contrast to other methods have extremely high cell densities. Although in load-bearing tissues with high amount of mineral components and noticeable mechanical properties use of this methods still looks uncertain, the ability to position aggregates of heterotypic cells with microscale precision (**Figure 2**) seems promising as an excellent alternative to bioprint complex tissues consisting variety of cells [49].

4. Manufacturing of scaffolds with 3-D printing technology

Researches aimed to investigate novel technologies for 3-D printing and introduced some novel methods including phase-separation, self-assembly, electrospinning, freeze drying, solvent casting/particulate leaching, gas foaming, and melt molding [52]. Using scaffolds, the architecture of native extracellular matrices can be mimicked at the nanoscale level and therefore provide the primary base for the regeneration of new tissue [53]. Originally, a "top-down" approach was used as a tissue engineering method for scaffold fabrication. In this method, cells are seeded onto a biodegradable and biocompatible scaffold and are predicted to migrate and fill the scaffold hence creating their own matrix. By using this technique, several avascular tissues such as bladder [54] and skin [55] have been engineered effectively. However, due to the limited diffusion properties of these scaffolds, this technique faces several challenges for fabrication of more complex tissues such as heart and liver [56]. Therefore, "bottom-up" methods have been developed to overcome this problem [57]. Bottom-up approaches include cell-encapsulation with microscale hydrogels, cell aggregation by self-assembly, generation of cell sheets, and direct printing of cells [58]. These complex tissue blocks can be assembled using various methods including microfluidics [59], magnetic fields [60], acoustic fields [61], and surface tension [62]. These methods are relatively easy and have provided a solid foundation for the fabrication of scaffolds. However, as mentioned previously, these conventional methods suffer from several limitations including inadequate control over scaffold properties such as pore size, pore geometry, distribution of high levels of interconnectivity, and mechanical strength. As such, it is necessary to develop technologies with sufficient control so as to design more intricate tissue-specific scaffolds. In addition, scaffolds can be coated using surface modification techniques (such as introducing functional groups) to enhance cell migration, attachment and proliferation. Three-dimensional printing allows scaffolds to become more precisely fabricated (similar to that of the computer-aided design (CAD)) with higher flexibility in the type of materials used to make such scaffolds. Three-dimensional printing uses an additive manufacturing process where a structure is fabricated using a layer-by-layer process. Materials deposited for the formation of the scaffold may be cross-linked or polymerized through heat, ultraviolet light, or binder solutions. Using this technology, 3-D printed scaffolds can be prepared for optimized tissue engineering [52].

For appropriate formation of tissue architecture, the seeding cells (often stem cells) require a 3-D environment/matrix similar to that of the ECM. The ECM acts as a medium to provide proteins and proteoglycans among other nutrients for cellular growth. The

ECM also provides structural support to allow for cellular functionality such as regulating cellular communication, growth, and assembly [63]. With this in mind, scientists and engineers originally attempted to replicate the ECM through conventional techniques, which consequently established a framework for using more advanced techniques, such as 3-D printing, to yield higher quality scaffolds. The 3-D printing technique can create defined scaffold structures with controlled pore size and interconnectivity and the ability to support cell growth and tissue formation [64–66]. The current methods for 3-D printing involve a CAD, which is then relayed to each 3-D printing system to "print" the desired scaffold structure. Through various 3-D printing technologies, discussed below, researchers are trying to fabricate biocompatible scaffolds that efficiently support tissue formation (**Table 3**).

5. Bioprinting advantages aiming for clinical use

The goal of tissue engineering is to create functional tissues and organs for regenerative therapies and ultimately organ transplantation/replacement. Trial and error was the long and tedious process mainly used to advance the field of regenerative medicine by clarifying the success of techniques.

Researchers needed to come up with a list of requirements in order to measure their successes or failures in tissue fabrication [48, 67]. This list was generated from the observations of natural human tissue.

As gold standard of fabricated tissues is to be as similar as possible to natural tissues in the human body in different parameters, then these fabricated tissues must:

1. Be able to integrate with naturally occurring tissue, and attach via microsutures, glues [68], or through cell adhesion [69–71].

2. Be capable of essential functions in vivo [48].

3. Become fully vascularized in order to sustain its functionality [68, 71].

Also, the printers used for tissue fabrication required standardization as well [67, 69].

1. The bioprinting machines required set extreme sterilization methods to eliminate unwarranted contamination with previously used materials or foreign matter from the environment.

2. The conditions for printing must be ideal for tissue fabrication, so factors such as humidity and temperature must be closely monitored.

3. Nozzle size and methods of delivery affect the viability of the materials being printed; therefore, there must be set ideals for delivery methods in relation to the various printing materials.

Printing method	Advantages	Disadvantages	Preclinical progress
Direct 3-D printing/ inkjet	• Versatile in terms of usable materials • No support is necessary for overhang or complex structures	• Potential toxicity (incompletely removed binders) • Low mechanical strength prints compared to laser sintering • Time consuming (post-processing)	• (Rat/bone) • (Rabbit/bone) • (Mouse/bone)
W/electrospinning			• (Mouse/cartilage)
Bioplotting	• Prints viable cells • Soft tissue applications	• Limitation on nozzle size* (*Must not be cytotoxic during processing) • Requires support structure for printing complex shapes	• (Rabbit/trachea) • (Rabbit/cartilage) • (Rat/cartilage) • (Mouse/cartilage) • (Mouse/tooth regeneration) • (Mouse/skin)
Fused deposition modeling	• Low cytotoxicity vs direct 3-D printing • Relatively inexpensive (printers and materials)	• Limitation on materials (often requires thermoplastics) • Materials used are nonbiodegradable • Requires support structure for overhangs and complex shapes • Post-processing may be necessary • Low Resolution	• (Swine/bone) • (Rat/bone)
Selective laser sintering	• Provides scaffolds with high mechanical strength • Powder bed provides support for complex structures • Fine resolution	• Limitation on materials (must be shrinkage and heat resistant) • Very high temp required (up to 1400°C) • Expensive and time consuming (processing and post processing)	• (Mouse/bone) • (Rat/heart) • (Rat/bone) • (Mouse/skin) • (Mouse/heart)

Printing method	Advantages	Disadvantages	Preclinical progress
Stereolithography	• Very high resolution • Speed of fabrication • Smooth surface finish	• Materials must be photopolymers • Expensive (two photon printers) • Support system is necessary for overhang and intricate objects	• (Rat/bone) • (Rabbit/trachea) • (Pig/tendon)
Electrospinning	• Speed of fabrication • Cell printing • Soft tissue engineering • Low shear stress (bioelectrospraying)	• Random orientation of fibers • Nonuniform pore sizes • High voltage (1–30 kV) requirements	• (Mouse/biocompatibility) • (Rat/bone) • (Rabbit/vascular tissue)
Indirect 3-D printing	• Good for prototyping/preproduction • Material versatility casting once mold is obtained	• Requires proprietary waxes for biocompatibility (wax printing) • Low accuracies/resolution • Mold required for casting • Long production times (mold → cast → processing → product)	• (Rat/bone) • (Mouse/tooth regeneration)

Table 3. Preclinical researches on various 3-D printing techniques for manufacturing scaffolds for tissue engineering [52].

As a result, researchers created a few methods of printing with the goal of finding a solution to the given problems for optimal tissue biofabrication [48, 68, 69]. Thermal inkjet bioprinting with bioink and direct-write bioprinting both make use of modified inkjet printers but with varied application techniques. Organ printing with tissue spheroids is the recent achievement of researches which seems promising to fabricate tissues directly. **Table 4** review advantages and disadvantages of all three common methods "Thermal Inkjet Bioprinting," "Direct-Write Bioprinting," and "Spheroid Organ Printing." Organ printing, otherwise known as the biomedical

Type of bioprinting	Method	Tissue characteristics	Note
Thermal inkjet bioprinting	• Bottom up • Layer-by-layer	• Avascular • Aneural • Alymphatic • Thin • Only nourishable via diffusion	"Bioink," which is a water-based liquid consisting of proteins, enzymes, and cells suspended in a media or saline.
Direct-write bioprinting	• Digital control of print. • Several printing units simultaneously. • Application of variety of materials simultaneously. • Faster turnaround time for printed products.	Possibility of printing tissues with different compositions.	• The bioink of direct-write printers may consist of hydro-gels of varying consistencies that are composed of agarose, alginate, collagen type I, and Pluronic F127. • This method categorized in pneumatic, mechanical, and a pneumatic-mechanical hybrid. It was concluded that the pneumatic systems work better with high viscosity materials, while mechanical systems are better suited in working with materials of low viscosity.
Spheroid organ printing	Spheroids are punched into "biopaper" which is a sprayed layer of hydrogel. Each spheroid is made of living cells, thereby creating a ball of "living materials" capable of self-assembly and self-fusion. Alternatively, the spheroids can be digitally placed, undergo self-assembly, and fuse without the use of hydrogel.	• Self-organization is defined as, "a process in which patterning at the global level of a system emerges solely from numerous interactions among the lower-level components of the system." • Self-assembly is defined to be, "the autonomous organization of components into patterns or structures without human intervention."	Researchers fabricated three types of spheroids to create a vascular tree: solid or nonlumenized spheroids, spheroids with one big lumen (mono-lumenized spheroids), and microvascularized tissue spheroids.

Table 4. 3-D bioprinting technique advantages and properties[67].

application of rapid prototyping, may be defined as additive layer-by-layer biomanufacturing of cells. Advantages of organ printing include its automated approach offering a pathway for a scalable and reproducible mass production of tissue-engineered products. This also allows the precise simultaneous 3-D positioning of several cell types, hence enabling the creation of tissue with a high level of cell density. Organ printing may be used to solve the problem of vascularization in thick tissue constructs, and moreover, this technology may be done in situ. Therefore, this emerging transforming technology has potential for surpassing traditional solid scaffold-based tissue engineering [72].

6. Current limitations

6.1. Vascularization

In order to create a complete and functional organ, the researchers must be able to create thick complex tissues with full vascularization containing lumens of various sizes, large vascular structures to microstructures, in order to sustain the surrounding organ tissue. The best way to achieve this type of vascularization is to fabricate the vascular system and tissue simultaneously, of which is easier said than done [48]. Thorough vascularization remains a common theme for current bioprinting limitations. Without a functional circulatory system, tissue constructs are limited to a means of diffusion for nutrition, which in itself is limited to just a few hundred microns [69].

Current methods of vascularization call for the infiltration of host microvessels into an implanted construct [67, 73, 74].

Yet, this strategy is lacking in control and specificity for the developing microvessels. The invading microvessels have a limited penetration depth which prevents the successful incorporation of the microvessels into larger layered constructs. Additionally, the penetration of the vascular system itself may result in a distortion of the region penetrated or in the destruction of the fabricated tissue altogether. For these reasons, it would be ideal to construct tissues with direct vessel in-growth, or vascularization created within the tissue itself, all before implantation.

6.2. Tissue components and costs

In addition to vascularization, native tissues contain unique cellular combinations and organizations. There is a need to develop techniques that mimic the complexity of native tissues in order to drive tissue recovery and replacement for medical applications [69]. With the production of organs such as kidneys, for example, at least one million glomeruli and nephrons would need to be generated. Not only would the fabrication be a massive undertaking but also the fabricated tissue would need to be scalable. Scalability of biofabricated tissues is not presently a reality. Yet, spheroids have shown promise toward being scalable with further development. Finally, another major limitation for the development of natural-like, fully functioning fabricated human tissue is economic [68]. This challenge must definitely be overcome if biofabrication technology is to allow the creation of a functional living human organ.

7. Future aspects of 3-D printing for regenerative medicine

In this chapter, we have illustrated current guiding principles for 3-D bioprinting in tissue fabrication, as well as recent advances and technological developments. The speed at which our knowledge has advanced with additive manufacturing and automated printing systems shows a promise to expand our basic science and engineering capabilities toward addressing health care problems. One of the significant developments in 3-D bioprinting is to manufacture cell microenvironments from molecular to macroscopic scales, which are requested and suitable for tissue engineering and regenerative medicine. As novel methods and technologies introduced in recent years for 3-D printing of biomaterials, promising overview of future appears to manufacture scaffolds for tissue engineering that reach the gold standards and also better comprehensions of stem cells microenvironments and interactions. By aid of various novel technologies, such as microfluidic systems [75, 76], biopatterning [77], and layer-by-layer assembly [76, 78], researchers are now able to biomanufacture microtissue constructs within scaffolds and even also within scaffold-free environments. Considering the great and enormous improvements of biomaterial for tissue engineering, in contrast, there are still certain challenges and difficulties that need more attention. Vascularization is one of the limitations which receive most of attentions [79, 80] due to the fact that this challenge leads to hypoxia, apoptosis, and immediate cell death. For resolving this issue and providing sufficient space for vascularization, researchers attempts to fabricate porous scaffolds [81], to provide sufficient space for vascularization. However, this approach cannot overcome the vascularization challenge completely due to the diffusion of cells and other materials into these porous structures [82]. Forming interconnected, well-defined vascular structures during biomanufacturing process seems to lead to resolving this difficulty and providing better results during process. Other issues that have to be noticed are mechanical strength and stability in 3-D tissue engineering which is one of the key requirements [83]. To be clear in regeneration of hard (e.g., bone) and soft (e.g., vascular grafts) tissues, modulus of elasticity is a crucial parameter that desires improvement [84–86]. Furthermore, the development of a totally closed bioprinting system that integrates printing and post-printing processes such as in-vitro culture and maturation of tissue constructs continues to be a challenge.

With advances in near future, which help finding solutions for the challenges mentioned above, bioprinting technologies will potentially help improvements of rapid clinical solutions and advances in medical implants. Further, we envision that the integration of cells and biomaterials through bioprinting with microfluidic technologies are likely to create unique microenvironments for various applications in cancer biology, tissue engineering, and regenerative medicine [87–91]. Additionally, developments on high-throughput biomanufacturing of 3-D architectures will pave the way for further advancements of in vitro screening and diagnostic applications, potentially enabling complex organ constructs. In the meantime, it is only the effective interplay of engineering concepts in combination with the well-established fundamentals of biology that will realize the true potential of this exciting area.

Author details

Seied Omid Keyhan[1,2,3,4], Hamidreza Fallahi[5], Alireza Jahangirnia[6],
Mohammad Taher Amirzade-Iranaq[7] and Mohammad Hosein Amirzade-Iranaq[8,9,10,11]*

*Address all correspondence to: h.amirzade@gmail.com

1 Department of Oral & Maxillofacial Surgery, Faculty of Dentistry, Birjand University of Medical Science, Birjand, Iran

2 Vice Presidential Organization of Technology of the Islamic Republic of Iran, Iran

3 Stem Cell & Regenerative Medicine Network, Shahid Beheshti University of Medical Sciences, Tehran, Iran

4 Cranio Maxillofacial Research Center, Tehran Dental Branch, Islamic Azad University, Tehran, Iran

5 Oral and Maxillofacial Surgery, Jundishapur University of Medical Sciences, Ahvaz, Iran

6 Oral and Maxillofacial Surgeon, Private Practice, Tehran, Iran

7 Biomaterial Medical Engineer, Department of Materials, Faculty of Materials Engineering, Islamic Azad University Najaf Abad Branch, Isfahan, Iran

8 Student Research Committee (SRC), Baqiyatallah University of Medical Sciences, Tehran, Iran

9 Universal Network of Interdisciplinary Research in Oral and Maxillofacial Surgery (UNIROMS), Universal Scientific Education and Research Network (USERN), Tehran, Iran

10 Student Research Committee, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

11 International Otorhinolaryngology Research Association (IORA), Universal Scientific Education and Research Network (USERN), Tehran, Iran

References

[1] Gibbs DM, Vaezi M, Yang S, Oreffo RO. Hope versus hype: What can additive manufacturing realistically offer trauma and orthopedic surgery? Regenerative Medicine. 2014;**9**(4):535-549

[2] Canstein C, Cachot P, Faust A, Stalder A, Bock J, Frydrychowicz A, et al. 3D MR flow analysis in realistic rapid-prototyping model systems of the thoracic aorta: Comparison with in vivo data and computational fluid dynamics in identical vessel geometries. Magnetic Resonance in Medicine. 2008;**59**(3):535-546

[3] Müller A, Krishnan KG, Uhl E, Mast G. The application of rapid prototyping techniques in cranial reconstruction and preoperative planning in neurosurgery. Journal of Craniofacial Surgery. 2003;**14**(6):899-914

[4] Hoy MB. 3D printing: Making things at the library. Medical Reference Services Quarterly. 2013;**32**(1):93-99

[5] Rengier F, Mehndiratta A, von Tengg-Kobligk H, Zechmann CM, Unterhinninghofen R, Kauczor H-U, et al. 3D printing based on imaging data: Review of medical applications. International Journal of Computer Assisted Radiology and Surgery. 2010;**5**(4):335-341

[6] Chae MP, Rozen WM, McMenamin PG, Findlay MW, Spychal RT and Hunter-Smith DJ (2015) Emerging applications of bedside 3D printing in plastic surgery. Front. Surg. 2:25. DOI: 10.3389/fsurg.2015.00025

[7] Mertz L. New world of 3-d printing offers "completely new ways of thinking": Q&A with author, engineer, and 3-d printing expert hod lipson. Pulse, IEEE. 2013;**4**(6):12-14

[8] Ibrahim AMS, Jose RR, Rabie AN, Gerstle TL, Lee BT, Lin SJ. Three-dimensional Printing in Developing Countries. Plastic and Reconstructive Surgery – Global Open. 2015;**3**(7):e443

[9] Chan HH, Siewerdsen JH, Vescan A, Daly MJ, Prisman E, Irish JC. 3D rapid prototyping for otolaryngology—head and neck surgery: Applications in image-guidance, surgical simulation and patient-specific modeling. PLoS One. 2015;**10**(9):e0136370

[10] Mendez BM, Chiodo MV, Patel PA. Customized "In-Office" three-dimensional printing for virtual surgical planning in craniofacial surgery. Journal of Craniofacial Surgery. 2015;**26**(5):1584-1586

[11] Cunningham LL, Madsen MJ, Peterson G. Stereolithographic modeling technology applied to tumor resection. Journal of Oral and Maxillofacial Surgery. 2005;**63**(6):873-878

[12] AlAli AB, Griffin MF, Butler PE. Three-Dimensional Printing Surgical Applications. Eplasty. 2015;**15**:e37

[13] Hull CW. Apparatus for Production of Three-Dimensional Objects by Stereolithography. Google Patents; 1986

[14] Dawood A, Marti BM, Sauret-Jackson V, Darwood A. 3D printing in dentistry. British Dental Journal. 2015;**219**(11):521-529

[15] Brix F, Hebbinghaus D, Meyer W. Verfahren und Vorrichtung für den Modellbau im Rahmen der orthopädischen und traumatologischen Operationsplanung. Röntgenpraxis. 1985;**38**:290-292

[16] Sinn DP, Cillo Jr JE, Miles BA. Stereolithography for craniofacial surgery. Journal of Craniofacial Surgery. 2006;**17**(5):869-875

[17] Mankovich NJ, Cheeseman AM, Stoker NG. The display of three-dimensional anatomy with stereolithographic models. Journal of Digital Imaging. 1990;**3**(3):200-203

[18] Suomalainen A, Stoor P, Mesimäki K, Kontio RK. Rapid prototyping modelling in oral and maxillofacial surgery: A two year retrospective study. Journal of Clinical and Experimental Dentistry. 2015;**7**(5):e605

[19] Barker T, Earwaker W, Lisle D. Accuracy of stereolithographic models of human anatomy. Australasian Radiology. 1994;**38**(2):106-111

[20] Frühwald J, Schicho KA, Figl M, Benesch T, Watzinger F, Kainberger F. Accuracy of craniofacial measurements: Computed tomography and three-dimensional computed tomography compared with stereolithographic models. Journal of Craniofacial Surgery. 2008;**19**(1):22-26

[21] Mazzoli A, Germani M, Moriconi G. Application of optical digitizing techniques to evaluate the shape accuracy of anatomical models derived from computed tomography data. Journal of Oral and Maxillofacial Surgery. 2007;**65**(7):1410-1418

[22] Mehra P, Miner J, D'Innocenzo R, Nadershah M. Use of 3-d stereolithographic models in oral and maxillofacial surgery. Journal of Maxillofacial and Oral Surgery. 2011;**10**(1):6-13

[23] Cohen A, Laviv A, Berman P, Nashef R, Abu-Tair J. Mandibular reconstruction using stereolithographic 3-dimensional printing modeling technology. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2009;**108**(5):661-666

[24] Mazzoni S, Marchetti C, Sgarzani R, Cipriani R, Scotti R, Ciocca L. Prosthetically guided maxillofacial surgery: Evaluation of the accuracy of a surgical guide and custom-made bone plate in oncology patients after mandibular reconstruction. Plastic and Reconstructive Surgery. 2013;**131**(6):1376-1385

[25] Eppley BL, Sadove AM. Computer-generated patient models for reconstruction of cranial and facial deformities. Journal of Craniofacial Surgery. 1998;**9**(6):548-556

[26] Gerstle TL, Ibrahim AM, Kim PS, Lee BT, Lin SJ. A plastic surgery application in evolution: Three-dimensional printing. Plastic and Reconstructive Surgery. 2014;**133**(2):446-451

[27] Chopra K, Gastman BR, Manson PN. Stereolithographic modeling in reconstructive surgery of the craniofacial skeleton after tumor resection. Plastic and Reconstructive Surgery. 2012;**129**(4):743e-745e

[28] Melchels FP, Feijen J, Grijpma DW. A review on stereolithography and its applications in biomedical engineering. Biomaterials. 2010;**31**(24):6121-6130

[29] Yan X, Gu P. A review of rapid prototyping technologies and systems. Computer-Aided Design. 1996;**28**(4):307-318

[30] Choi JW, Kim N. Clinical application of three-dimensional printing technology in craniofacial plastic surgery. Archives of Plastic Surgery. 2015;**42**(3):267-277

[31] Seied Omid Keyhan, Sina Ghanean, Alireza Navabazam, Arash Khojasteh and Mohammad Hosein Amirzade Iranaq (2016). Three-Dimensional Printing: A Novel Technology for Use in Oral and Maxillofacial Operations, A Textbook of Advanced Oral and Maxillofacial Surgery Volume 3, Prof. Mohammad Hosein Kalantar Motamedi (Ed.), InTech, DOI: 10.5772/63315

[32] Annabi N, Tamayol A, Uquillas JA, Akbari M, Bertassoni LE, Cha C, et al. 25th anniversary article: Rational design and applications of hydrogels in regenerative medicine. Advanced Materials. 2014;**26**(1):85-124

[33] Bakarich SE, Gorkin III R, in het Panhuis M, Spinks GM. Three-dimensional printing fiber reinforced hydrogel composites. ACS Applied Materials & Interfaces. 2014;**6**(18): 15998-16006

[34] Bertassoni LE. Bioprinting of human organs. Australasian Science. 2015;**36**(3):34

[35] Bertassoni LE, Cardoso JC, Manoharan V, Cristino AL, Bhise NS, Araujo WA, et al. Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels. Biofabrication. 2014;**6**(2):024105

[36] Woodruff MA, Hutmacher DW. The return of a forgotten polymer—polycaprolactone in the 21st century. Progress in Polymer Science. 2010;**35**(10):1217-1256

[37] Hutmacher DW, Sittinger M, Risbud MV. Scaffold-based tissue engineering: Rationale for computer-aided design and solid free-form fabrication systems. TRENDS in Biotechnology. 2004;**22**(7):354-362

[38] Xavier JR, Thakur T, Desai P, Jaiswal MK, Sears N, Cosgriff-Hernandez E, et al. Bioactive nanoengineered hydrogels for bone tissue engineering: A growth-factor-free approach. ACS Nano. 2015;**9**(3):3109-3118

[39] Michna S, Wu W, Lewis JA. Concentrated hydroxyapatite inks for direct-write assembly of 3-D periodic scaffolds. Biomaterials. 2005;**26**(28):5632-5639

[40] Tarafder S, Dernell WS, Bandyopadhyay A, Bose S. SrO- and MgO-doped microwave sintered 3D printed tricalcium phosphate scaffolds: Mechanical properties and in vivo osteogenesis in a rabbit model. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2015;**103**(3):679-690

[41] Wang Y, Azaïs T, Robin M, Vallée A, Catania C, Legriel P, et al. The predominant role of collagen in the nucleation, growth, structure and orientation of bone apatite. Nature Materials. 2012;**11**(8):724-733

[42] Sousa F, Evans JR. Sintered hydroxyapatite latticework for bone substitute. Journal of the American Ceramic Society. 2003;**86**(3):517-519

[43] Morissette SL, Lewis JA, Cesarano J, Dimos DB, Baer T. Solid freeform fabrication of aqueous alumina–poly (vinyl alcohol) gelcasting suspensions. Journal of the American Ceramic Society. 2000;**83**(10):2409-2416

[44] Tevlin R, McArdle A, Atashroo D, Walmsley G, Senarath-Yapa K, Zielins E, et al. Biomaterials for craniofacial bone engineering. Journal of Dental Research. 2014;**93** (12):1187-1195

[45] Hutson CB, Nichol JW, Aubin H, Bae H, Yamanlar S, Al-Haque S, et al. Synthesis and characterization of tunable poly (ethylene glycol): Gelatin methacrylate composite hydro-gels. Tissue Engineering Part A. 2011;**17**(13-14):1713-1723

[46] Gao G, Schilling AF, Yonezawa T, Wang J, Dai G, Cui X. Bioactive nanoparticles stimu-late bone tissue formation in bioprinted three-dimensional scaffold and human mesen-chymal stem cells. Biotechnology Journal. 2014;**9**(10):1304-1311

[47] Müller WE, Schröder HC, Feng Q, Schlossmacher U, Link T, Wang X. Development of a morphogenetically active scaffold for three-dimensional growth of bone cells: bio-silica–alginate hydrogel for SaOS-2 cell cultivation. Journal of Tissue Engineering and Regenerative Medicine. 2015;**9**(11):E39-E50

[48] Mironov V, Visconti RP, Kasyanov V, Forgacs G, Drake CJ, Markwald RR. Organ printing: Tissue spheroids as building blocks. Biomaterials. 2009;**30**(12):2164-2174

[49] Ikeda E, Morita R, Nakao K, Ishida K, Nakamura T, Takano-Yamamoto T, et al. Fully functional bioengineered tooth replacement as an organ replacement therapy. Proceedings of the National Academy of Sciences. 2009;**106**(32):13475-13480

[50] Norotte C, Marga FS, Niklason LE, Forgacs G. Scaffold-free vascular tissue engineering using bioprinting. Biomaterials. 2009;**30**(30):5910-5917

[51] Tabatabaee FS MS, Gholipour F, Khosraviani K, Khojasteh A..Craniomaxillofacial bone engineering by scaffolds loaded with stem cells: A Systematic review. Journal of Dental School. 2012;**30**(2):115-131

[52] Do A-V, Khorsand B, Geary SM, Salem AK. 3D Printing of scaffolds for tissue regeneration applications. Advanced Healthcare Materials. 2015;**4**(12):1742-1762

[53] Wei G, Ma PX. Nanostructured biomaterials for regeneration. Advanced Functional Materials. 2008;**18**(22):3568-3582

[54] Korossis S, Bolland F, Southgate J, Ingham E, Fisher J. Regional biomechanical and histological characterisation of the passive porcine urinary bladder: Implications for augmentation and tissue engineering strategies. Biomaterials. 2009;**30**(2):266-275

[55] Groeber F, Holeiter M, Hampel M, Hinderer S, Schenke-Layland K. Skin tissue engineering — In vivo and in vitro applications. Advanced Drug Delivery Reviews. 2011;**63**(4-5): 352-366

[56] Huang G, Wang L, Wang S, Han Y, Wu J, Zhang Q, et al. Engineering three-dimensional cell mechanical microenvironment with hydrogels. Biofabrication. 2012;**4**(4):042001

[57] Nichol JW, Khademhosseini A. Modular tissue engineering: Engineering biological tissues from the bottom up. Soft Matter. 2009;**5**(7):1312-1319

[58] Napolitano AP, Chai P, Dean DM, Morgan JR. Dynamics of the self-assembly of complex cellular aggregates on micromolded nonadhesive hydrogels. Tissue Engineering. 2007;**13**(8):2087-2094

[59] Chung SE, Park W, Shin S, Lee SA, Kwon S. Guided and fluidic self-assembly of microstructures using railed microfluidic channels. Nature Materials. 2008;**7**(7):581-587

[60] Xu F, Wu CaM, Rengarajan V, Finley TD, Keles HO, Sung Y, et al. Three-dimensional magnetic assembly of microscale hydrogels. Advanced Materials. 2011;**23**(37):4254-4260

[61] Xu F, Finley TD, Turkaydin M, Sung Y, Gurkan UA, Yavuz AS, et al. The assembly of cell-encapsulating microscale hydrogels using acoustic waves. Biomaterials. 2011;**32**(31):7847-7855

[62] Kachouie NN, Du Y, Bae H, Khabiry M, Ahari AF, Zamanian B, et al. Directed assembly of cell-laden hydrogels for engineering functional tissues. Organogenesis. 2010;**6**(4):234-244

[63] Geckil H, Xu F, Zhang X, Moon S, Demirci U. Engineering hydrogels as extracellular matrix mimics. Nanomedicine. 2010;**5**(3):469-484

[64] Lee K-W, Wang S, Lu L, Jabbari E, Currier BL, Yaszemski MJ. Fabrication and characterization of poly (propylene fumarate) scaffolds with controlled pore structures using 3-dimensional printing and injection molding. Tissue Engineering. 2006;**12**(10):2801-2811

[65] Xiaoming Li, Rongrong Cui, Lianwen Sun, et al., "3D-Printed Biopolymers for Tissue Engineering Application," International Journal of Polymer Science, vol. 2014, Article ID 829145, 13 pages, 2014. DOI:10.1155/2014/829145

[66] Bose S, Vahabzadeh S, Bandyopadhyay A. Bone tissue engineering using 3D printing. Materials Today. 2013;**16**(12):496-504

[67] Patra S, Young V. A review of 3D printing techniques and the future in biofabrication of bioprinted tissue. Cell Biochemistry and Biophysics. 2016;**74**(2):93-98

[68] Cui X, Boland T, D D'Lima D, K Lotz M. Thermal inkjet printing in tissue engineering and regenerative medicine. Recent Patents on Drug Delivery & Formulation. 2012;**6**(2): 149-155

[69] Chang CC, Boland ED, Williams SK, Hoying JB. Direct-write bioprinting three-dimensional biohybrid systems for future regenerative therapies. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2011;**98**(1):160-170

[70] Jakab K, Damon B, Marga F, Doaga O, Mironov V, Kosztin I, et al. Relating cell and tissue mechanics: Implications and applications. Developmental Dynamics. 2008;**237**(9): 2438-2449

[71] Dean DM, Morgan JR. Cytoskeletal-mediated tension modulates the directed self-assembly of microtissues. Tissue Engineering Part A. 2008;**14**(12):1989-1997

[72] Ozbolat IT, Yu Y. Bioprinting toward organ fabrication: Challenges and future trends. IEEE Transactions on Biomedical Engineering. 2013;**60**(3):691-699

[73] Barralet J, Gbureck U, Habibovic P, Vorndran E, Gerard C, Doillon CJ. Angiogenesis in calcium phosphate scaffolds by inorganic copper ion release. Tissue Engineering Part A. 2009;**15**(7):1601-1609

[74] Borselli C, Ungaro F, Oliviero O, d'Angelo I, Quaglia F, La Rotonda MI, et al. Bioactivation of collagen matrices through sustained VEGF release from PLGA microspheres. Journal of Biomedical Materials Research Part A. 2010;**92**(1):94-102

[75] Nakao Y, Kimura H, Sakai Y, Fujii T. Bile canaliculi formation by aligning rat primary hepatocytes in a microfluidic device. Biomicrofluidics. 2011;**5**(2):022212

[76] Chang R, Emami K, Wu H, Sun W. Biofabrication of a three-dimensional liver microorgan as an in vitro drug metabolism model. Biofabrication. 2010;**2**(4):045004

[77] Gurkan UA, El Assal R, Yildiz SE, Sung Y, Trachtenberg AJ, Kuo WP, et al. Engineering anisotropic biomimetic fibrocartilage microenvironment by bioprinting mesenchymal stem cells in nanoliter gel droplets. Molecular Pharmaceutics. 2014;**11**(7):2151-2159

[78] Snyder J, Hamid Q, Wang C, Chang R, Emami K, Wu H, et al. Bioprinting cell-laden matrigel for radioprotection study of liver by pro-drug conversion in a dual-tissue microfluidic chip. Biofabrication. 2011;**3**(3):034112

[79] Rupnick MA, Panigrahy D, Zhang C-Y, Dallabrida SM, Lowell BB, Langer R, et al. Adipose tissue mass can be regulated through the vasculature. Proceedings of the National Academy of Sciences. 2002;**99**(16):10730-10735

[80] Langer RS, Vacanti JP. Tissue engineering: The challenges ahead. Scientific American. 1999;**280**:86-89

[81] Hutmacher DW. Scaffold design and fabrication technologies for engineering tissues—State of the art and future perspectives. Journal of Biomaterials Science, Polymer Edition. 2001;**12**(1):107-124

[82] Henze U, Kaufmann M, Klein B, Handt S, Klosterhalfen B. Endothelium and biomaterials: Morpho-functional assessments. Biomedicine & Pharmacotherapy. 1996;**50**(8):388

[83] Hockaday L, Kang K, Colangelo N, Cheung P, Duan B, Malone E, et al. Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds. Biofabrication. 2012;**4**(3):035005

[84] Mironov V, Boland T, Trusk T, Forgacs G, Markwald RR. Organ printing: Computer-aided jet-based 3D tissue engineering. TRENDS in Biotechnology. 2003;**21**(4):157-161

[85] Prestwich GD. Hyaluronic acid-based clinical biomaterials derived for cell and molecule delivery in regenerative medicine. Journal of Controlled Release. 2011;**155**(2):193-199

[86] Guven S, Lindsey JS, Poudel I, Chinthala S, Nickerson MD, Gerami-Naini B, et al. Functional maintenance of differentiated embryoid bodies in microfluidic systems: A platform for personalized medicine. Stem Cells Translational Medicine. 2015;**4**(3):261-268

[87] Rizvi I, Gurkan UA, Tasoglu S, Alagic N, Celli JP, Mensah LB, et al. Flow induces epithelial-mesenchymal transition, cellular heterogeneity and biomarker modulation in 3D ovarian cancer nodules. Proceedings of the National Academy of Sciences. 2013;**110**(22):E1974-E1983

[88] Luo Z, Güven S, Gozen I, Chen P, Tasoglu S, Anchan RM, et al. Deformation of a single mouse oocyte in a constricted microfluidic channel. Microfluidics and Nanofluidics. 2015;**19**(4):883-890

[89] Anchan R, Lindsey J, Ng N, Parasar P, Guven S, El Assal R, et al. Human IPSC-derived steroidogenic cells maintain endocrine function with extended culture in a microfluidic chip system. Fertility and Sterility. 2015;**104**(3):e73

[90] Gurkan UA, Tasoglu S, Akkaynak D, Avci O, Unluisler S, Canikyan S, et al. Smart interface materials integrated with microfluidics for on-demand local capture and release of cells. Advanced Healthcare Materials. 2012;**1**(5):661-668

[91] Gurkan UA, Anand T, Tas H, Elkan D, Akay A, Keles HO, et al. Controlled viable release of selectively captured label-free cells in microchannels. Lab On a Chip. 2011;**11**(23): 3979-3989