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REVIEW

Nanoscale 3D Bioprinting for Osseous Tissue Manufacturing

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Abstract: 3D printing, as a driving force of innovation over many areas, brings numerous manufacturing methods together from the macro to nano scales. New revolutionary materials (such as polymeric materials and natural biomaterials) can be produced into unique 3D printed nanostructures. The morphology and functionality of various 3D printing methods as well in vitro and in vivo results of their use towards regenerating bone are discussed in this review. This review further focuses nano scale 3D bioprinting technology for bone tissue engineering, mainly including recent progress in research on technical materials and methods, typical applications, and crucial achievements; explaining the scientific and technical challenges for bone tissue fabrication; and describing micro-nano scale 3D printing application prospects, development directions, and trends for the future for this field to realize its full potential.

Keywords: orthopedic, nanoparticles, nanotextured, bone, advance manufacturing

Background

Printing techniques for various applications have been under constant development for thousands of years. Woodblock printing was invented in China, although researchers have not found an exact date with concrete evidence when this technique was originally discovered.[1] However, woodblock printing was widely used in the Tsin Dynasty (303–379 AD) to print calendars.[1] In 15th Century Europe, contemporary shapes for printing, wide spread publishing and the book trade began to gradually emerge with revolutionary impacts on religion, society, educational materials, industry, etc.[2,3] In the early 1980s, three dimensional (3D) printing was first established from early additive manufacturing and materials by Hideo Kodama using photo-harden thermoset polymers.[4,5] In 1986, Charles Hull first introduced 3D printing in the form of stereolithography. Stereolithography enabled engineers to make 3D solid objects by successively printing curable materials, layer-by-layer, using ultraviolet light.[6] Sacrificial resin molds were next created through this mechanism to form 3D scaffolds for biomaterials and regenerative medicine.[7] The straightforward printing of biomaterials into 3D scaffolds is extremely promising with potential for transplantion into patients.[8] In 2014, the global 3D bioprinting market size was estimated at $487 million, and it is expected to grow to $1.82 billion by 2022.[9] By combining 3D printing, nanotechnology, cell biology and materials science, bone tissue engineering exhibits enormous prospects for future development. This promise comes from both an easier method to produce implants as well as its use in personalized medicine with nanoscale structure.

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It is predicted that the annual cost of bone repairs will be greater than $5 billion in the United States by 2020 indicating a growing need for bone related biomaterials.[10] In general, orthopedic tissue engineering is a sophisticated process involving remodeling of the bone by osteoprogenitor cells during cell proliferation, differentiation, and matrix formation.[11] Typically, bone scaffolds are made of adsorptive biodegradable materials that provide sufficient mechanical backing and many of the necessary environmental cues in the period of reconstruction and regeneration of injured or infected bone.[12] 3D printing technologies in orthopedic applications have grown tremendously in recent years owing to an excellent capability to precisely produce porous scaffolds. Besides the advantages mentioned before, other benefits include both shape and chemistry controllability, and interconnected porosity.[13] In addition, nano/micro scale 3D printing technologies have the same advantages with many artificial bone tissue engineering biomaterials, including bone grafts or other biomaterials, but do not have to be obtained from donors or other parts of the body. Furthermore, they also eliminate the risk of tissue rejection and disease transfer from donor tissue. Today, numerous 3D printing biomaterials for bone tissue engineering are either on the market or are in advanced lab testing. For example, Dal et al mentioned porous ceramic scaffold prototypes for bone tissue engineering applications manufactured by using 3D printing from powder materials.[14] Tarafder et al developed 3D interconnected macroporous tricalcium phosphate (TCP) scaffolds combined with microwave sintering to obtain samples with high mechanical strength.[15] The surface of 3D printed scaffolds is extremely important in the functions of cells involved in bone remodeling, in particular, osteoclast-like cells. Detsch's group compared cell proliferation and differentiation with different scaffold surfaces made of pure hydroxyapatite (HA), β-TCP, as well as a biphasic mixture of HA and TCP.[16]

Compared with conventional materials, dimension controlled nanoscale materials may be more efficient materials at stimulating new bone formation due to their ability to adsorb specific proteins and enhance adsorbed protein bioactivity to influence cellular functions.[11] Due to quantum effects and their large surface-area-to-volume ratios, nanomaterials with a variety of sizes, morphologies, and surface modifications have unique physical, mechanical, optical, chemical, and biological properties, which are providing specific advantages to the field of tissue engineering.[17]

The improvement of nanoscale 3D bioprinting in mechanical properties and tissue regeneration due to greater select cell adhesion, can be optimized through the use of nanoscale features.[18] These nanomaterials including nanofibers, nanotubes, nanoparticles, and hydrogels have been designed and tested as scaffolds to resemble the extracellular matrix (ECM) of bone (and other tissues) and replace defective tissues. For example, nano-hydroxyapatite (n-HA) was used for in vivo 3D bioprinting for implantation into a mouse calvaria critical size defect by Keriquel.[19] Another group conjugated n-HA with printed bone scaffolds containing not only a bone generation supporting complex but also interconnected channels to mimic micro-vasculature.[20] This structure supported osteogenic differentiation and bone regeneration of mesenchymal stem cells, along with vascular cell ingrowth, which is important for providing nutrients and removing cellular waste.[20] Chitosan/nHA powders have also been utilized to print honeycomb geometry scaffolds with high porosity, high strength, and biological compatibility.[21] Besides, scaffolds coated with collagen/n-HA,[22] alginate/n-HA,[23] gelatin/n-HA,[24] and alginate/gelatin interpenetrated with homogeneous nano apatites have been fabricated via 3D printing combined with in situ mineralization.[25] In addition, Webster et al designed and developed novel 3D printed polylactic acid scaffolds with cold atmospheric plasma, which promoted both osteoblast and hMSC attachment and proliferation due to nanoscale features.[26]

Over history, utilizing completely regenerated bone in transplants has been a dream of human beings. From the first-generation to the current third-generation biomaterials,[8] new improved biomaterials have been developed.[8] Due to the continuous development of materials, it has been hard to fully distinguish between bone replacement and bone regeneration. Numerous biomaterials possess functionality to promote bone regeneration, however, it is still a significant challenge to regenerate bone tissue over a short period of time without any side-effects.[27] In this review, micro-nano scale 3D bioprinting for bone tissue engineering is discussed, mainly including recent progress in research on materials and methods, typical applications, and crucial achievements. Furthermore, the state-of-the-art scientific and technical challenges for bone tissue fabrication are explained. Finally, we describe micro-nano scale 3D printing application prospects, development directions, and trends for the future to realize the full potential of 3D printing in orthopedics.

3D Printing Techniques

Introduction

Bone scaffolds can be prepared by gas generation or solvent casting,[28–30] freezing/freezing-drying techniques,[31,32] and phase separation.[33–36] However, one common drawback of these approaches is that the size and shape of the resulting pores cannot be accurately restricted. Another technique, additive manufacturing (AM), has become more popular in recent decades. AM is a technique that allows for targeted materials to grow on top of each other. Specifically, Hull was the first person who reported and developed a well-known stereolithography process in 1986, which is one of the typical techniques used in AM. Since then, AM techniques have attracted large attention and have been developed considerably and improved. Compared to traditional scaffold fabrication methods, the advantages of AM include an automated manufacturing process and precise geometry control.

Thus far, many AM approaches have been reported or modified to form porous scaffolds. Based on the materials and the manufacturing processes, typical AM methods can be categorized into non-biological 3D printing and biological 3D printing. Non-biological printing techniques include fused deposition modeling (FDM), lamination object manufacturing (LOM), stereo lithography (SLA), selective laser sintering (SLS), selective laser or electron beam melting (SLM or EBM), etc. On the other hand, biological scaffold printing techniques, for example, inkjet bioprinting and laser-assisted bioprinting, are commonly reported in the literature. Among these biological 3D printing techniques, most of them exhibit an ability to manufacture scaffolds that can be potentially utilized for human bone regeneration. Table 1 presents the comparison of such approaches for bone scaffold manufacturing. It is notable that not all of these AM methods may be applied to biological materials. For example, electron beam melting requires the use of extremely high temperature, which limits its application in biological 3D printing.

Fused Deposition Modeling

The most common method applied in AM is called fused deposition modeling (FDM) due to its acceptable cost, high fabrication speed, and ease of operation. The FDM method has been reviewed by Mohamed et al[37] in the literature. Target materials, usually polymer filaments, are heated into a semi-liquid state at the nozzle and then jetted onto a substrate or previously printed layers. Solidification occurs after being exposed to the air at room temperature, resulting in 3-D structures. Such a process is highly dependent on the thermoplasticity of the filament itself. Commonly used materials include acrylonitrile butadiene styrene (ABS), polycarbonate (PC), and a mixture of ABS and PC. The geometry of the manufactured structure can be accurately managed by

Table 1 Comparasion of Approaches for Bone Scaffold Manufacturing

	Methods	Special Characteristics	Features	References
Traditional Manufacturing	Solvent casting	Material dissolved in organic solvent	Cannot be accurately controlled	26–28
	Freezing-drying techniques	Low temperature required		29,30
	Phase separation	Two phases required		31–34
Additive Manufacturing (AM)	Fused deposition modeling (FDM)	Common materials include ABS, PC, mixture of ABS and PC	Non- biological manufacturing	35–39
	Lamination object manufacturing (LOM)	Successive layers are initially heated		
	Stereo lithography (SLA)	UV light or electron-beam is used to start reaction		40–43
	Selective laser sintering (SLS)	Metal or alloy powders melting by laser or electron beam		44–50
	Electron beam melting (EBM)			44,45,47
	Selective laser (SLM)			46–50
	Inkjet bioprinting	Same principle with traditional inkjet printers	Biological manufacturing	7,51,52,69,80,85
	Laser-assisted bioprinting	Nozzle-free deposition		7,53,55

the movement of a fabrication platform (Z direction) and jet nozzle (X-Y direction) controlled by a computer aided design (CAD) data file. The surface quality of the obtained materials as a function of FDM process parameters has been investigated by a number of researchers. It was found that parameters, such as layer thickness, road width, speed deposition, raster angle, and deposition temperature have an effect on surface roughness when ABS polymeric materials were used.[38–40] Although a lot of work has been done to optimize the quality of the porous materials obtained by FDM (for example, mechanical properties, surface roughness, accuracy, etc.), there are several concerns for the FDM method. For example, porous polymer poly(ε-carrolactone) (PCL) scaffolds with bioresorbility have been made using FDM, but could not meet the in vivo culture requirements regarding channel size and porosity.[41]

Stereolithography

Stereolithography (SLA), which has been utilized to solidify 3D scaffolds by a polymerization process, is one of the earliest methods used in AM dating back to at least 1986. Typically, a UV light or electron-beam is used to initiate the polymerization chain reaction on a layer or monomer solution. Once one layer is completely solidified, a platform lowers in the vertical direction (Z-direction) by a small distance and continues with a new layer. These steps repeat until a new model has been finalized. Compared with other AM techniques, SLA exhibits excellent reproducibility and high accuracy.[42,43] However, the manufacturing speed of this technique is relatively slow, and the selection of an initial substrate or polymer solution is highly limited because it is expected to be UV light sensitive. In addition, due to the complexation of the polymerization process, the solidification step needs to be carefully controlled. Schuster et al[45] structured 3D materials by SLA from gelatin-based monomers in the presence of an additive, which are promising as bone replacement materials. Cell culture experiments confirmed that the monomers used had very low cytotoxicity, which can be considered negligible. Qiu et al[44] reported new porous scaffold chitosans with light curability and solubility synthesized by SLA, which could be potentially used for human bone tissue repair.

Selective Laser and Electron Beam Melting

Selective laser melting (SLM) and electron beam melting (EBM) are relatively new and rapid prototyping tools for the additive manufacturing of metals or alloys with CAD. Metal, alloy, polymer, or ceramic powders from a powder feed forms a compact layer that is typically several powder particles thick, and then are selectively melted by a focused laser or electron beam.[46,47] Melted powders rapidly solidify in an inert environment such as pure argon (SLM and EBM) or nitrogen (SLM). Co-based (Co29Cr6Mo[48]), Ni-based (Ni21Cr9Mo4Nb[48]), and Ti-based (Ti[49] and Ti6Al4V[50–52]) metallic materials have been prepared in this way as reported in the literature.

Inkjet Bioprinting

Inkjet printing is one of most conventional approaches to be utilized for nanoscale materials. Although it has the same principle as traditional drop-on-demand printers, biological materials such as cells and hydrogels[7,53] supplant the ink inside the cartridge, and the paper is replaced by a one degree-of-freedom[27,53,54] (in the z-axis) computer controlled printing bed. Thermal force or piezoelectricity[27] are two typical extrusion forces of the printing head, which is essentially controlled by customized CAD files, to accurately load the "ink material" to the substratum (Figure 1A). A 3D positioning system is constituted by the printing bed and a two degree-of-freedom[53] (in x and y axes) printing head (Figure 1B). It is notable that the 3D positioning system can be utilized not only in the inkjet printing system but also for other approaches.

Laser-Assisted Bioprinting

Laser-assisted bioprinting is an application of laser-induced forward transfer (Figure 2).[7] This nozzle-free deposition technique was first utilized in metal manufacturing, and it has been employed in organ and tissue fabrication.[46,47] Numerous biomaterials can be applied as the "ink material", which includes peptides, genes, and cells.[55–57] A typical laser-assisted bioprinter consists of the energy absorbing layer, the laser pulse, and the donor slide.[27] During the process of printing, the energy absorbing layer generates pressure by receiving energy from the laser, and then transmits the pressure to the donor side and propels it to the substrate. The resolution of laser-assisted bioprinting is impacted by numerous factors, including the laser intensity, the surface tension, the wettability of the substrate, the air gap between the ribbon and the substrate, and the thickness and viscosity of the biological layer.[7]

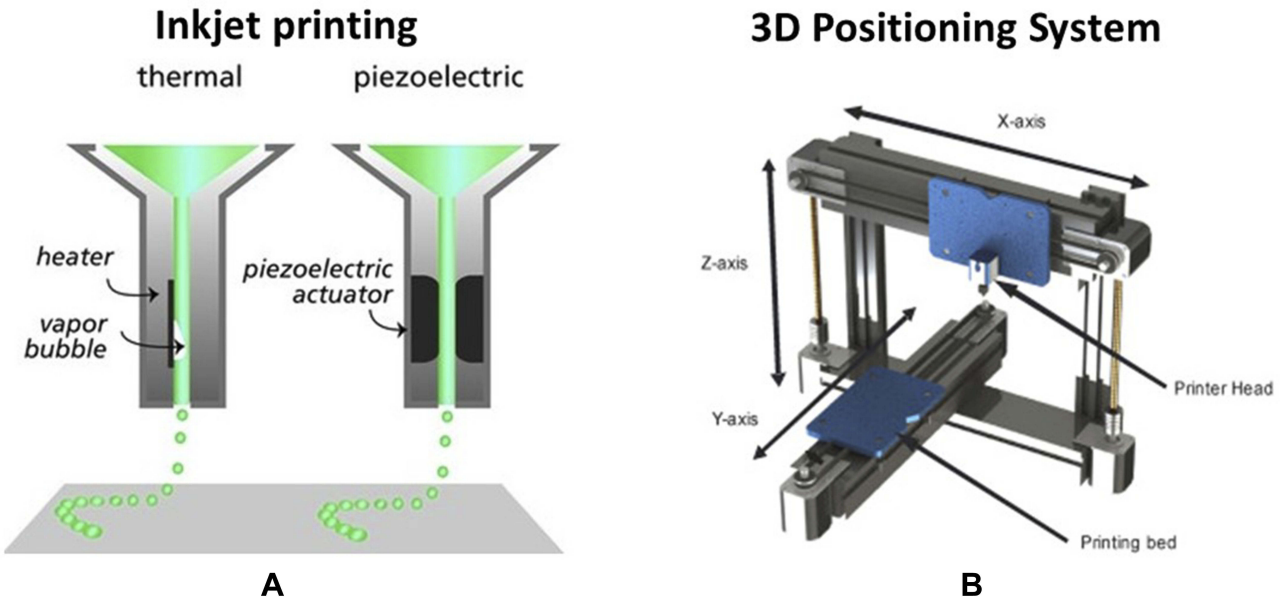

Figure 1 (**A**) Adapted with permission from Malda J et al. 25th Anniversary Article: Engineering Hydrogels for Biofabrication. *Adv Mater.* 2013;25(36):5011–5028. © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.[27] describes the mechanism of an inkjet bioprinter driven by a thermal and piezoelectric force. (**B**) Adapted from Munaz et al. Three-dimensional printing of biological matters. *J Sci Adv Mater Devices*. 2016;1(1):1–17. *Copyright © 2016 Elsevier.*.[53] describes the mechanism of the 3D positioning system.[27,53]

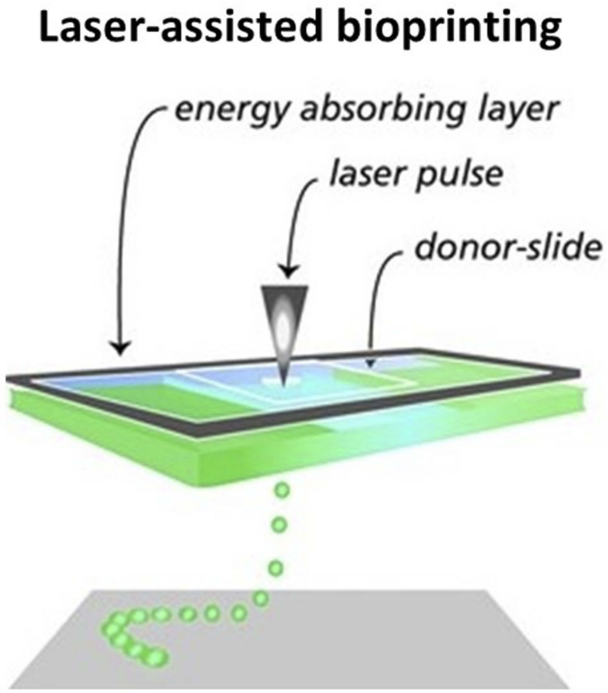

Figure 2 Adapted with permission from Malda J et al. 25th Anniversary Article: Engineering Hydrogels for Biofabrication. *Adv Mater.* 2013;25(36):5011–5028. © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim; showing the principle of a laser-assisted bioprinter.[27]

3D Printing Materials

Introduction

Biomaterials used in bone tissue engineering should have properties (mechanical strength, structure, chemistry, etc.) close to human tissue. It has been demonstrated that bone is a specialized form of connective tissue, consisting of osteoblasts, osteoclasts, proteins, and inorganic components.[58–60] Thus far, materials that have been developed as bone tissue engineering scaffolds include polymers, ceramics and natural-bone-like composites, which will be further described below in the context of 3D printing. Lately, nanomaterials (or materials with at least one dimension less than 100 nm) have been extensively studied for orthopedic applications as they mimic the natural nanometer components of bone. Table 2 presents a comparison of materials used in bone scaffold manufacturing.

Polymeric Materials

Polymers, with their diversity and ease in 3-D printing operations, have attracted great attention in orthopedic applications. This area has been well reviewed by Ligon et al.[61] SLA and SLS are suitable tools for the deposition of polymers during 3-D printing. Compared to SLS, however, SLA is quite limited because it requires light-sensitive polymers and the thermomechanical properties of the obtained scaffolds still needs to be improved. Through SLS, many polymeric materials, such as polycarbonate[62] and polypropylene[63] can be prepared. One ideal polymeric material that has been widely utilized in tissue engineering is

Table 2 Comparison of Materials for Bone Scaffold Manufacturing

	Typical Materials	Special Characteristics	Manufacturing Methods	References
Polymeric Materials	Polystyrene, Polycarbonate, Polyamides, Polyesters	Potential ability to promote bone growth and inhibit infection	Chemical etching, Laser etching	7,9,32,93,94,96
Ceramic Materials	Pure hydroxyapatite, Pure tricalcium phosphate and their composites	Chemical composition close to human bone tissue; Good biocompatibility	High temperature Heating treatment	14,59–63,88
Metallic Materials	Titanium (Ti) and its alloys	High mechanical strength	Electron beam melting (EBM)	47–50,64–68, 71,72,74
Natural Biomaterials	Hydrogels, Tissues, Organs, Peptides, DNAs, Cells	Increase tissue forming cell function; Decrease infection and inflammation	Inkjet bioprinting Laser-assisted bioprinting	7,55,57,69,76,87

polyesters. Polyesters, synthesized by a condensation polymerization process between two functional groups, a carboxylic acid group (-COOH) and a hydroxyl group (-OH), are able to degrade into natural metabolites through hydrolysis in the human body. Post-degrading small molecules, typically lactic acid and glycolic acid, are endogenous to human metabolism. Commonly used polyester species, such as poly (lactic acid) (PLA)[64] and poly(ε-caprolactone) (PCL),[65] have been reported in the literature. Biologically-inspired nanostructures have been implemented into/onto polymers after 3D printing via chemical etching, laser etching, and many other techniques demonstrating the ability to promote bone growth and inhibit infection without using drugs.

Ceramic Materials

Commonly used ceramic materials in orthopedics include pure hydroxyapatite (HA), tricalcium phosphate (TCP) and their composites. HA has been recognized as a promising material to fabricate bone tissues due to its chemical composition, which is close to human bone tissue and has good biocompatibility. Researchers have reported the manufacturing of porous ceramic scaffolds by using 3D printing techniques, which was designed and built in cooperation with the Generis GmbH company, followed by a high temperature heating treatment.[66] In this case, ceramic powders and modified HA powders, were mixed with a polymer-based binder solution and printed layer by layer. The resulting structures reduced inner channel and wall thickness dimensions. The biocompatibility of 3D printed structures by using pure HA and TCP was tested by Warnke et al.[67] It was demonstrated that HA based structures possess more biocompatibility compared with TCP. Besides that mentioned above, the mixture of HA and TCP has also been studied. For example, Suwanprateeb et al[68] investigated the properties of a manufactured HA/apatite–wollastonite ceramic glass as a factor of heating temperature and time. They observed that the lowest porosity, greatest strength, and flexibility were obtained at a temperature of 1300 °C for 3 hrs. In addition, the newly customized calcium-phosphate layer when immersed into simulated body fluid had greater biocompatibility with human osteoblasts. Balcik et al[69] found a greater mean radiological grade of healing and bonding to bone with HA/TCP (60/40) ceramics than that of pure HA ceramics. Ramay and Zhang synthesized HA/TCP porous scaffolds by a gel casting method and found that the resulting mechanical properties were promoted by the presence of HA and increased with HA concentration.[70] Moreover, nanoscale surface features are easy to create on 3D printed ceramics since nano particles or nanostructures can be easily incorporated during 3D printing.

Metallic Materials

With good structural and mechanical compatibility for load bearing applications, metals and metal alloys have been considered promising materials for bone tissue engineering applications. As the human body is known to be a corrosive medium with various pH and electric potentials, a biocompatible type of metallic material with high corrosion resistance is highly desired. Therefore, compared to other metallic biomaterials, elemental titanium (Ti) and its alloys exhibit excellent features for corrosion resistance[71,72] and biocompatibility. However, the compressive strength of bones lies in a range of 10–30 GPa, while this number in Ti is 110 GPa leading to an insufficient match of mechanical

properties between bone and metallic implants. To solve this problem, one useful strategy is to introduce pores into metallic structures for reducing the Young's modulus of metallic materials.[73] For example, it has been reported that the low Young's modulus (5.3 GPa) of Ti was fabricated when using a porosity of 78%.[74] Oh et al[75] fabricated porous Ti compacts with a porosity ranging from 5.0 to 37.1 vol% by a powder sintering method. In addition, they found that the bending strength obtained at a porosity of 30 vol% was very close to human cortical bone. Controllable micron structured pore sizes, specifically for Ti_6Al_4V, have been prepared by electron beam melting (EBM), which melted Ti_6Al_4V powders in a layer by layer fashion.[52] Part of the fabrication process was completed in a vacuum environment to avoid introducing impurities coming from oxygen. The obtained porous structures with pore sizes ranging from 765 μm to 1960 μm and the mechanical strength of these models had a porosity of 50–70%, which met requirements for craniofacial applications. Moreover, anodization has been widely used to create nanoscale features on Ti based implants to improve bone growth, decrease inflammation, and inhibit infection without using drugs.

Natural Biomaterials

Typically used natural materials in 3-D printing include certain hydrogels, tissues, organs, peptides, DNAs, and cells. It is notable that the mechanical construction and mechanical properties should be well considered during the process of material selection. Commonly used or commercialized hydrogels have been used to fabricate 3-D scaffoldings via a modified inkjet bioprinter, as reported by Murphy.[76] In their studies, parameters that can affect the quality of the obtained scaffolding (such as gelation time, swelling, etc.) were carefully investigated and their contraction, stability and biocompatibility were compared. 3-D printing of pluripotent embryonal carcinoma cells by a laser-assisted bioprinting method at the micro-scale was demonstrated by Ringeisen et al[57] where nearly 100% of cell viability post transfer was observed using a live/dead assay. In terms of nanotexturing, natural materials inherently possess nanostructured features to increase tissue forming cell function and decrease infection and inflammation.

Applications

Introduction

Bone fractures, osteoporosis, and osteoarthritis are the most common clinical orthopedics manifestations, which lead to approximately 2.2 million bone tissue transplants each year.[77] Numerous materials such as polymeric, ceramic, metallic, and natural materials can be manufactured by 3D printing approaches. Before those materials can be utilized in bone tissue engineering, various factors in the bone healing process, which include biocompatibility, mechanical properties, and vesicular structure, should be considered thoroughly. However, the requirements can vary depending on the application. Figure 3 presents an example to achieve 3D printing of bone tissue, adapted from ref 77; Figure 3(B and C) shows a preliminary biocompatibility test with the growth morphology of MC3T3-E1 cells adherent to tricalcium phosphate/tetralcium phosphate (TCP/TTCP) scaffolds treated with an osteogenesis inducing medium.[13,77,78]

Biocompatibility

An ideal bone scaffold material should neither suppress the activity of healthy osteocytes, osteoblasts, and osteoclasts or should show any significant cytotoxicity in both the process of transplant surgery and during the post-surgery period.[78] Several additional requirements applied to the materials include an osteogenesis-induced effect

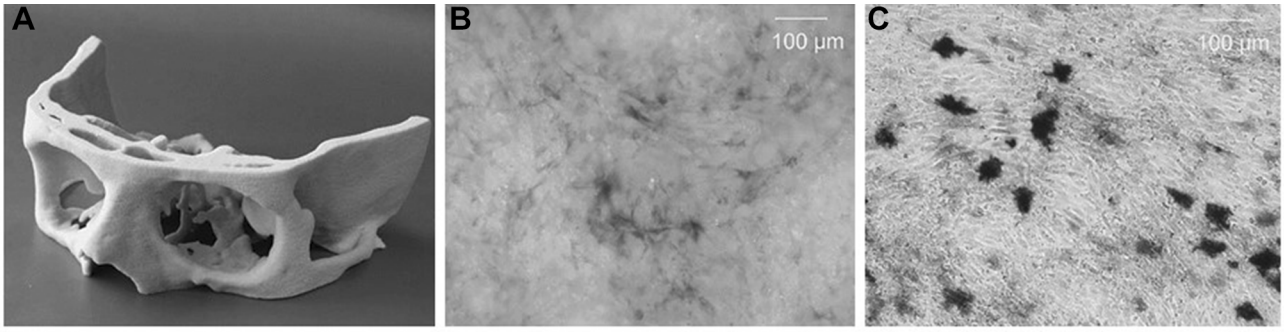

Figure 3 (**A**) 3D printed cranial segment; (**B**) MC3T3-E1 cells producing alkaline phosphatase (AP) after 21 days; and (**C**) MC3T3-E1 cells cultured on tricalcium phosphate/tetracalcium phosphate (TCP/TTCP) scaffolds after 21 days. Reprinted by permission from RightLink: Springer, *J Eur Soc Biomater.* Khalyfa A et al. Copyright, 2007.[77]

that may accelerate the adhesion and proliferation of osteoblasts or mesenchymal stem cells (MSCs), both of which help to form structurally supportive extracellular matrices (ECM). At the nanoscale, cells can robustly adhere and proliferate among a 3D nano environment composed of nanofibers. This is due to a larger specific surface area responsible for this situation, which can enhance the adsorption and bioactivity of proteins as well as cell adhesion and growth.[79]

Mechanical Properties

As a required property for bone transplants, mechanical strength is a major challenge for the 3D printing technique applied to porous scaffolds and osseous tissue manufacturing. For instance, the mechanical strength of current 3D printed bone implants may not meet the requirements for bone transplants. However, in the ideal condition, the scaffold material should not only match the mechanical strength of the surrounding tissue but provide sufficient nutrition transportation. Some studies have clearly indicated that mechanical strength is strongly controlled by the pore volume of the printed materials. More specifically, the strength of the scaffold and the pore volume demonstrate a negative correlation. In addition, several parameters (e.g., temperature, material formulation, etc.) can alter the mechanical strength of 3D printed scaffolds. For instance, a mixture of β-TCP/TTCP sintered at 1400 °C can increase its mechanical strength while the TTCP/calcium sulfate dihydrate composite after sintering can cause weaker mechanical properties.[77] In terms of an investigation by Aston et al, a variety of materials assembled at the nanoscale, which include nanofibers, nanopillars, nanoparticles, and nanocomposites, show potential for meeting the mechanical strength requirements for bone tissue scaffolds.[40]

Vesicular Structure

A vesicular structure is an essential factor for scaffold materials utilized in bone repair. One previous study by Rouwkema et al mentioned that scaffolds should have effective pore diameters beyond 250 μm to optimize the rate of capillary ingrowth. This ensures the transportation of nutrients and oxygen due to diffusion limitations from the capillary.[80] Another study by Murphy et al indicated that scaffold materials with a mean pore diameter of 325 μm can optimize cell inflitration in scaffolds and in part accelerate bone regeneration.[81] Furthermore, a series of studies have shown that scaffolds with multiple porous diameters demonstrate a greater result than those with identical porous sizes.[82,83]

Challenges and Prospects

Due to the large market size in both the current period and what is predicted, 3D printed bone, as a major branch and application of biological additive manufacturing, has a favorable prospect. One of the most significant applications of nanoscale 3D printed bone is in the bone transplant industry.[7,84,85] For example, CAD designed 3D printed polymethyl-methacrylate (PMMA) implants have been utilized to repair skull defects between 2009 to 2011. Among a total of 16 patients, 15 people recovered completely, and only one postoperative infection occurred after a 28 month follow-up. Compared to the traditional surgical methods, this approach reduced the surgical time and decreased the risks of complications.[85] Technically, both mature in vitro osteogenic cells and artificial materials can be utilized in 3D bioprinting.[86–88] However, artificial materials suffer several drawbacks.[86,89] For instance, it is difficult to build microvasculature in artificial materials; meanwhile, the vessel structure is crucial to supply nutrients for cells.[90,91] Hence, with the advantages of self-healing, remodeling, and maintainance, the ideal materials utilized in bone transplant are the patients own bone and cells (osteoclasts and osteoblasts).[13] Moreover, 3D printed tissues and organs can be applied for drug discovery, chemical analysis, and basic research.[7] For instance, 3D printed calcium phosphate (CaP) ceramics have been utilized in evaluating bone regenerative potential, biocompatibility, and toxicology in vitro.[92] Our group used cold atmospheric plasma (CAP) to nanomodify 3D printed polylactic acid (PLA) scaffolds, which has been widely investigated for anti-cancer and anti-bacterial applications.[26] Generally, it is reasonable to predict that those applications demonstrate an extremely high potential for future research.

As a newly developed biological manufacturing approach, numerous technical problems still exist for 3D bone tissue bioprinting. Hydrogels, as the primary scaffold materials for printing cells, have low mechanical strength,[93] which limit their applications in 3D printed bone tissue.[88] The other technical problem of 3D printing is resolution. Several major approaches[7] can only control the resolution at the microscale. For example, inkjet bioprinters[94] can achieve a resolution of 50 μm and microextrusion can control the resolution to 5μm, but it is hard to produce resolution at the nanometer scale using current technology. It is notable that the size of a single cell is from 1 μm to 100 μm, and current technologies cannot perfectly print

nanostructures, such as extracellular matrices, between the cells. Advanced 3D printing approaches, such as the IBM output printers with 3D patterning technology, can increase the resolution to 15 nm.[95] However, this technology currently is not applied to bioprinting.

Each technical method brings both advantages and blemishes. The main 3D printing approaches, including FDM, LOM, SLA, SLS, EBM, SLM, inkjet bioprinting, and laser-assisted bioprinting,[7] have specific requirements in their raw materials. For example, the operating requirements of SLS technology refers to nano-scale metals and metal alloy powders. Thus, it is difficult to apply this technology to bone tissue manufacturing directly. EBM is rarely applied to biological nanoscale manufacturing, rather it is preferably used to manufacture metallic nanostructures. Biological materials can be utilized in inkjet bioprinting and laser-assisted biopointing directly, but the mechanical strength of those materials often do not meet the requirements for transplants. Generally, due to those specific requirements, a perfect individual technology to fit all of the environments necessary for 3D bone printing is not available. A foreseeable speculation for the future of 3D printing techniques is to combine different technologies for both 3D printing and 3D bioprinting.

In conclusion, the field of 3D printing bone scaffolds is currently experiencing rapid development. As the approach directly utilizes biological manufacturing, inkjet bioprinting and laser-assisted bioprinting can be considered as an upgrade for the drop-on-demand printer at the nanoscale. Theoretically, osseous tissue is made of cancellous bone and cortical bone. Yet for accurate duplication, both structures must be simultaneously printed since they undergo dynamic remodeling, maturation, differentiation, and resorption. Using artificial materials and cells to achieve the function and structure for bone tissue implants is an exceptionally complex engineering problem. In the future, we believe 3D bioprinting approaches can meet the requirements to achieve this goal. To do so, we not only need integration among 3D printing techniques, but also synergy between 3D printing techniques and nanoscale operational methods and materials.[26] For mechanistic studies, further concerns (such as neural and vascular tissue regeneration in bones and other organs) need to be considered.[96] The mechanical analysis of the scaffold and intracellular substances should also be performed using CAD and other technique in the modeling of 3D printing bone tissue. Moreover, an interdisciplinary engineering theorem (including mechanics and dynamics) should be utilized in such nanoscale mechanism research for 3D printed bone structures.

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Disclosure

The authors report no conflicts of interest in this work.

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