3D Printing of Personalized Artificial Bone Scaffolds

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Abstract

Additive manufacturing technologies, including three-dimensional printing (3DP), have unlocked new possibilities for bone tissue engineering. Long-term regeneration of normal anatomic structure, shape, and function is clinically important subsequent to bone trauma, tumor, infection, nonunion after fracture, or congenital abnormality. Due to the great complexity in structure and properties of bone across the population, along with variation in the type of injury or defect, currently available treatments for larger bone defects that support load often fail in replicating the anatomic shape and structure of the lost bone tissue. 3DP could provide the ability to print bone substitute materials with a controlled chemistry, shape, porosity, and topography, thus allowing printing of personalized bone grafts customized to the patient and the specific clinical condition. 3DP and related fabrication approaches of bone grafts may one day revolutionize the way clinicians currently treat bone defects. This article gives a brief overview of the current advances in 3DP and existing materials with an emphasis on ceramics used for 3DP of bone scaffolds. Furthermore, it addresses some of the current limitations of this technique and discusses potential future directions and strategies for improving fabrication of personalized artificial bone constructs.

Introduction

BONE DEFECTS RESULTING from severe trauma, nonunion after fracture, tumor removal, infection, or congenital abnormalities are often too large to heal on their own and can lead to long-term deformity such as limb shortening, leaving patients with reduced bone structure and function. Autografts are considered the clinical gold-standard treatment for bone defects because they include a scaffolding structure along with cells and other biologics from the same patient. However, donor-site morbidity, limited availability for harvesting, requirement of intraoperative

modification, and differences in structure of bones from different sites of the body suggest the need for alternative methods.^{1,2} Structural allografts provide scaffolds with a range of different shapes and sizes, but are associated with limited revascularization² and risks of infection, immunological rejection,³ and long-term mechanical failure. Sintered blocks of calcium phosphates (CaP) such as tricalcium phosphate (TCP) and hydroxyapatite are used as bone substitute materials due to their chemical similarity to the organic apatite crystals of bone.³ However, they may require intraoperative modifications and the ability to fabricate microstructures is

limited using traditional manufacturing techniques.³

Because of the above drawbacks of allografts and autografts, CaP cements have been investigated for application as bone graft substitutes due to their injectability and ability to harden at body temperature.⁴ Although CaP cements are osteoconductive (when the bone graft material serves as a scaffold for new bone growth), there are still crucial issues such as weak cohesion of solid-liquid phase, intrinsic microporosity, and disintegration upon contact with blood or body fluids that need to be addressed to satisfy clinical needs.⁵ Furthermore, with

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injectable CaP pastes it is difficult to control porosity, surface topography, and shape of the graft. It is also challenging to create a composite graft material having mechanical properties similar to cortical or cancellous bone.

Another concern in developing efficacious bone graft substitutes or a scaffold is that significant variation in bone anatomy exists between patients. Furthermore, defect shape and size will be variable depending on the type of defect.^{6,7} These anatomical characteristics must be considered during graft fixation and design in order to improve graft osseointegration with host tissue. This suggests the need for using additive manufacturing (AM) technology along with computer-aided design (CAD) so that bone grafts or scaffolds with complex shapes, identified in patients via medical imaging techniques such as computed tomography (CT) and magnetic resonance imaging, can be manufactured.^{8,9} These grafts or scaffolds could then be used in biomedical applications ranging from customized medical implant design to tissue engineering.^{10,11} AM also has the distinct advantage of enabling scaffolds or artificial constructs to be built with predefined micro- as well as macrostructures.¹² Microstructure of constructs is also important because the pore size, orientation, and surface chemistry on the micron-scale dictate the extent and nature of vascular and bone tissue in-growth.

There are several different AM techniques, such as three-dimensional printing (3DP),^{13,14} selective laser sintering,^{12,15,16} sheet lamination,¹² and fused deposition modeling,^{15,16} that could be applied to generate 3D scaffolds (Fig. 1). O'Brien et al.¹⁷ provide a concise overview of the application of these different AM techniques for fabrication of tissue-engineered scaffolds in the fields of bone, osteochondral, neural, and vascular tissue regeneration. Table 1 summarizes comparisons between scaffolds generated using various AM techniques along with their relative advantages and disadvantages. Among these AM techniques, there has emerged great interest in 3DP to manufacture bone scaffolds. This is because 3DP, unlike other AM techniques that use

Figure 1. Schematic comparing various 3DP modalities discussed in this article.

Stereolithography uses light (UV) to cross-link photopolymer liquids that polymerizes layer-by-layer by immersion of a platform downward into the liquid. Fused deposition modeling, on the other hand, involves deposition of a melted thermoplastic (filaments) by extrusion through a nozzle. Selective laser sintering modality uses long-wavelength lasers (or a high-energy light source) to fuse beads of material one layer at a time, whereas powder 3DP does the same using a binder solution that locally hardens the powder bed where the binder is deposited. Ink-jet 3DP involves direct printing of liquid (polymer, cells, and biomolecules) using a nozzle and a print head in order to fabricate tissue engineering constructs. 3DP, three-dimensional printing.

extrusion, photopolymerization, high temperatures, or laser-assisted sintering for a layer-by-layer creation of 3D scaffolds, is a direct deposition process¹⁷ (Fig. 1 and Table 1). Unlike other methods, 3DP enables fabrication of heterogeneous tissue constructs composed of deposited cells, growth factors, extracellular matrix molecules, and the biomaterial of interest.¹⁸ 3DP is also a powder-based fabrication method using an ink-jet print head that prints binders on to loose powders in a powder bed. This allows the manufacture of personalized 3D models guided directly from computer data.¹⁹ For instance, a patient's CAD data can be used for fabrication of customized implants without the need of making a mold. Here we describe 3DP procedures, materials used, technical possibilities, and limitations of this technique. The purpose of this commentary is to provide the 3DP community a concise introduction to AM-based bone tissue engineering, including processes and materials, and how bone regenerative medicine may be

optimized through control of scaffold features such as surface topography.

Powder-Based 3DP

Materials and Printing Process

The 3DP method considered here is a powder-based ink-jet printing technique.²⁰ This technology is useful for printing bone scaffolds because a variety of powders (including metal and ceramic) and binders can be used. A typical 3DP procedure is divided into four steps. First, a homogenous powder bed surface is created by spreading fine powder granules using a set of rollers.¹⁶ Flowability of the powder is critical for the powder to spread, and is dependent on particle size, shape, and surface roughness.¹⁵ The size of the powder granules determines the smoothness and thickness of the powder bed formed, which in turn affects the resolution of the final printed construct. High flowability

Table 1. Examples of several additive manufacturing techniques used to fabricate scaffolds for bone tissue engineering

AM technology	Printing materials	Advantages	Disadvantages	References
Powder three-dimensional printing (binder solution)	PCL Hydroxyapatite HA/TCP HA/collagen Ceramics (calcium phosphates) Polymers Composites	Direct printing Wide range of material choice Cost effective	Lower green strength Postprocessing technique restricts biomolecule incorporation	13–14,23–24,35–37, 39–40
Ink-jet bioprinting (direct liquid printing)	Polymers (hydrogels) Living cells Proteins and biomolecules (collagen)	Processing conditions milder Allows for incorporation of biomolecules (proteins) and living cells (multiple cell types)	Limiting choice of biomaterials High required cell density	17,18
Fused deposition modeling (heated extrusion)	Thermoplastic polymers (PCL, poly-lactic acid, etc.) TCP	No platform or support needed Higher mechanical strengths of printed scaffold Fast and inexpensive	Material restrictions due to need for melting (require filaments of thermoplastic polymers) High temperatures Cannot incorporate biomolecules/cells Difficult to replicate geometries, low resolution Not suitable for musculoskeletal applications	15–17
Selective laser sintering (laser source)	Polymers Ceramics	Higher resolution No postprocessing required Higher mechanical properties	Requires long-wavelength or high-energy source (cannot print cells) Resolution depends on laser beam diameter Slow process, expensive Restricted to stiff materials	12,15–17
Stereolithography (UV/photopolymerization)	Polymers (photocrosslinkable)	Higher resolution	Only applicable with photopolymers Support structure required Use of UV light source restricts incorporation of biomolecules and living cells	12

Summary of different biomaterials used along with advantages and disadvantages between various additive manufacturing (AM) techniques.

and finer size granules permit formation of thin powder layers enabling higher resolution.¹⁹ However, fine powder can agglomerate due to attractive interactions between spherical particles (van der Waals forces)²¹ that can dominate gravitational forces and reduce flowability, resulting in poor compaction and unacceptable powder bed recoating. However, Butscher et al.²¹ also subjected their fine powder to plasma treatment with monomer hexamethyldisiloxane, which deposited tiny point-like nanostructures on the powder surface that acted as spacers

between the nanoparticles, increased the interparticle distance, reduced van der Waals forces, and improved flowability. Thus, a trade-off between flowability and resolution is unavoidable. Seitz et al.²² recommends using spherical granules (e.g., CaP) of mean diameter below 100 μm to ensure good resolution. Butscher et al.²¹ conducted a systematic evaluation of powder properties such as powder size and flowability in order to better understand the effect of these characteristics on printability of CaP. They fabricated and tested CaP powders

of 6, 18, 29, 35, and 50 μm size. Their results revealed that it was difficult to print with particles that were either too small or too large (6 or 50 μm) due to issues with low flowability and powder bed stability, respectively. They concluded that an optimal particle size range for getting good printability was between 20 and 35 μm .

In the second 3DP step, the print head sprays binder solution droplets on the powder bed in several passes guided by a CAD file. The final part consists of

material only at these specific locations. The binder solution can be an organic or aqueous solution (e.g., phosphoric acid²³ or dextrin²⁴) that can not only wet the surrounding powder, but also locally harden the wetted area.^{25,26} Binder drop volume and wettability are important parameters to consider before printing. Binder wettability influences the green strength (the initial strength after printing but before postprocessing steps) of the printed object, and is directly related to the powder particle surface energy, chemistry, and binder viscosity. Excessive wetting characteristics can lead to binder droplet spreading to a larger area than specified by the CAD file, resulting in poor resolution.¹⁹ Additionally, plasma treatment can impact wetting characteristics of the finer particles allowing binder droplets to displace them.²⁷ Printing thinner layers utilizes higher shear forces for recoating that require powder bed stabilization. This necessitates applying water moisture to the top powder layer leading to rearrangement of fine powders due to capillary effects.²⁷

In the third step, following binder deposition, the printed 2D layer is allowed to dry. In this step, a new layer of powder is also rolled over the printed layer following drying, in order to form a powder bed for the next binder deposition. The above three steps are repeated iteratively until the final 3D part is printed in a layer-by-layer manner.

The fourth and last step is de-powdering of the 3D printed part in order to remove any loose powder from the printed body. Postprocessing steps such as sintering¹⁹ and polymer infiltration^{15,28} have also been applied in the case of ceramic 3DP to improve mechanical properties, for example, compressive strength, of the printed part or to form a polymer-ceramic composite better mimicking bone's natural structure. Sintering at high temperatures, however, produces significant shrinkage of the printed part. This must be compensated for by increasing dimensions in the initial CAD model,²⁴ as shrinkage may change the desired resolution.

A range of materials, including polymers, ceramics, and composites, can be used for 3DP. Polymeric materials can be

synthetic (e.g., polylactic acid) or naturally occurring (starch, dextrose, collagen, etc.). Synthetic polymers have better mechanical properties, quality control, and degradation rates compared to natural polymers. However, they lack integrin-binding ligands and have hydrophobic surfaces resulting in lower cell proliferation,²⁹ which may necessitate their surfaces to be modified with receptors or hydrophilic coatings. Natural polymers are hydrophilic and can be used with water-based binders, whereas synthetic polymers require organic solvents like chloroform.¹⁹ Collagen is a main organic constituent of bone, and collagen scaffolds demonstrate excellent biological performance *in vivo* due to their high porosity, permeability, and biocompatibility. However, collagen scaffolds are weak and compliant.²⁹

Due to their excellent biocompatibility and osteoconductivity, CaP ceramic powders, with acid binders such as phosphoric acid/citric acid, are used widely in bone tissue engineering.^{13,14,23,30} There are two main approaches to using ceramic powders in the context of 3DP. In the first approach, sacrificial polymeric binders (that pyrolyze upon sintering)¹⁹ are used to improve green strength. In the second, the binder dissolves ceramic particles and forms new crystals that then interdigitate, forming stiffer ceramic networks. Composite materials such as combinations of ceramic (TCP), Bio-Glass, or polymers (natural polymers like collagen) have also been studied for use in 3DP. Composites can be formed either during printing using a polymeric binder with ceramic powder³¹ or by initially starting with a ceramic/polymer powder blend³² and an acid binder. However, powder and binder materials need to be selected with care as free radical initiators after printing can compromise biocompatibility.³³

In Vitro and In Vivo Testing of 3DP Bone Scaffolds

Synthetic bone grafts with the greatest potential to be efficacious are three-dimensional, biocompatible, and bioresorbable. Grafts should possess sufficient mechanical properties and yet be porous, to permit the flow of nutrients, vascularization, and bone ingrowth.^{1,16,33,34}

3DP allows manufacturing bone scaffolds with scalability, different anatomical geometries, and porosity. Calcium phosphate bind to living bone, giving these materials advantages as bone scaffold material. Klammert et al.³⁵ fabricated brushite (di CaP dihydrate) and monetite (di CaP anhydrous) 3D scaffolds to demonstrate the capability of 3DP for reconstruction of cranial defects and concluded that 3DP implants complied with the geometric requirements and provided an adequate fit. These investigators fabricated anatomically shaped scaffolds using CAD files generated by scanning a human cadaver skull having specific cranial defects. Tada et al.³⁶ fabricated HA-TCP implants for reconstruction of facial deformities by utilizing three-dimensional patient CT data that were transferred to create a life-sized CAD model of the defect, which in turn was used for shaping the artificial HA-TCP bone implant. They concluded that anatomically shaped templates and implants helped in optimizing the implant design and resulted in better contouring in cases with complex defects. Temple et al.³⁷ demonstrated feasibility of 3DP polycaprolactone (PCL) scaffolds with varying pore diameters having the shape of human mandibular and maxillary bones. Grayson et al.³⁸ successfully developed decellularized bone scaffolds that were shaped based on human temporomandibular joints, were seeded with human mesenchymal stem cells, and were provided appropriate bioreactor systems for the maturation of the graft before implantation.

Seitz et al.^{13,14,39} investigated the feasibility of fabricating 3DP HA scaffolds with complex internal structures and high resolution, using a water-soluble polymer binder (Schelofix) and sintering. They demonstrated that fabrication of cylindrical scaffolds with inner channel dimensions down to 450 μm was possible and allowed for osteointegration.¹³ Additionally, osteoblastic MT3T3-E1 cells attached, proliferated, and maintained their morphology on 3DP HA scaffolds.¹⁴ Another study³⁹ looking at biocompatibility of 3DP HA, TCP, and bovine HA blocks found, using human periosteal cells, that 3DP TCP and bovine HA blocks were biocompatible. These findings are important for bone

augmentation as periosteal cells are directly in contact with biomaterials in many situations. Castilho et al.²³ fabricated 3D biphasic CaP (BCP) scaffolds using a hydraulic setting reaction of HA-TCP powders incorporating phosphoric acid and posttreatment with polylactic-co-glycolic acid solution. They demonstrated that osteoblastic MG63 cell proliferation was greater on BCP composites compared to pure HA or TCP scaffolds. Becker et al.²⁴ fabricated 3DP HA and TCP blocks, as well as a bovine HA blocks with a central channel, using dextrin as a binder solution for intramuscular bone induction in a rat model. They found that HA and TCP blocks induced new bone formation demonstrating *in vivo* biocompatibility.

More recently, Inzana et al.⁴⁰ fabricated a CaP and collagen composite 3D scaffold using phosphoric acid binder to optimize the cytocompatibility and material parameters of 3DP ceramics. Using a murine segmental defect model, new bone formation that proceeded primarily through the intramedullary canal was demonstrated. They also demonstrated periosteal bone formation that incorporated the degrading scaffold material. The biocompatibility and resorbability of 3DP scaffolds, using pure HA, β -TCP, and a mixture of HA and TCP, was also studied using pre-osteoclastic RAW264.7 cells.⁴¹ RAW264.7 differentiated into osteoclast-like cells that resorbed CaP surfaces, but large lacunae formation was observed only on the surface of biphasic HA-TCP composites. This illustrates the influence of phase composition on resorption of ceramic 3D scaffolds.⁴¹ Furthermore, Konopnicki et al.⁴² fabricated 3DP 50% PCL and 50% TCP composite scaffolds seeded with porcine bone marrow progenitor cells (pBMP) and implanted them for 8 weeks into mandibular defects in minipigs. Unseeded scaffolds were used as controls. They found that TCP/PCL scaffolds seeded with pBMPs provided good bone penetration into the scaffold. They also observed angiogenesis at the center of the construct and around newly formed bone. These studies demonstrate the feasibility of using 3D ink-jet printing for fabricating bone scaffolds and also demonstrate their *in vitro* and *in vivo* biocompatibility.

Limitations and Future Prospects

With AM techniques, the overall resolution achievable with current systems and the range of useable materials for developing prototypes are two limiting characteristics. From an engineering perspective, overcoming low mechanical strength, limited resolution, and slow degradation of 3DP structures are major challenges in developing porous ceramic and composite scaffolds for bone substitutes. Poor mechanical properties can render the ceramic 3DP scaffolds unsuitable for high-load-bearing applications. 3DP HA scaffolds impregnated with bis-GMA⁴³ and TCP-sintered scaffolds³¹ were shown to improve mechanical properties. A 10-fold increase in compression strength (76 MPa) of 3DP TCP and tetra CaP (TTCP) scaffolds has been reported by Khalyfa et al. with sintering as compared to untreated scaffolds (0.7 MPa).²⁸ They also infiltrated the sintered scaffolds with bismethacrylated oligolactide macromer (DLM-1), containing 10 wt% of 2-hydroxyethyl methacrylate as comonomer and observed increases in the compression strength to 54 MPa as compared to untreated scaffolds (slightly lower than sintered scaffolds). Gbureck et al.³³ reported an increase in compressive strength of biphasic TCP scaffolds from 0.9 to 8.7 MPa (30 wt% acid concentration) with increasing concentrations of phosphoric acid binder. They also observed a fourfold increase in compressive strength from 5.3 MPa to more than 22 MPa, with 20 wt% acid concentration, after repeated postprinting hardening in diluted phosphoric acid.

However, with sintering the minimum pore size achieved thus far, without affecting the resolution of the printed ceramic, is 300 μm .²⁸ Also, one of the outcomes of sintering is nonuniform shrinkage, which makes it difficult to mimic cortical and trabecular bone structure as porosities in the scaffold will change during sintering. Most polymeric binders work well in improving strength and ductility in the powder-binder composite materials; however, polymeric binders are capable of producing residues that might not be easily removed during

the postprocessing steps and may result in *in vivo* cytotoxicity. Thus, selection of a more biocompatible binder-powder combination is required. Use of polymeric binders that can be resorbed during the postprocessing steps could be one way to address this problem. Composites of ceramics using resorbable glass materials to enhance mechanical properties could be another potential solution. The composite scaffold approach is promising for generation of stronger, more ductile, ceramic scaffolds, but differences among cortical and trabecular bone structures still require functionally graded materials, with local variations in material composition and mechanical properties, to be developed.

Micro- and nanoscale features of a scaffold may also have important effects on osseointegration of the scaffold. For instance, osteoblastic and preosteoblastic cells have been shown to respond *in vitro* to specific nanotopographical features that replicate bone's native surface structure.^{44,45} Similar features were associated with improved healing of a critical-sized defect *in vivo*.⁴⁶ Therefore, it may be advantageous to replicate in scaffolds the nanotopographical features of bone's native surface. However, scaffolds with nanotopographical features require higher spatial resolution than current 3DP can generally provide. One approach to improving the resolution of 3DP would be to use fine powder granules with higher flowability, and include an optimal binder solution and binder drop volume to avoid aggregation of finer powder particles that would result in a lower resolution.

Another approach to increasing 3DP resolution would be to incorporate particles of a nanoscale size into the 3DP "ink" to create nanoscale patterns. This approach is based on previous work showing that specific nanotopographies (60–80 nm but not 15–25 or over 100 nm), fabricated from HA, increase adhesion, proliferation, and osteoblastic differentiation of mesenchymal stem cells.⁴⁶ These nanotopographies refer to a mixed and variable topography, containing bumps, islands, and fractal patterns, as shown in Figure 2. The 25–30 nm coating had primary islands (900 nm in diameter), and the 50–60 nm coatings had secondary islands (200 nm in

Figure 2. Schematic of the different HA nanopatterns (25–30, 50–60, and 100–120 nm) showing the varied fractal patterns and islands. Secondary island formation was observed with 50–60 nm topography, while tertiary islands were observed on the 100–120 nm topography.

diameter) in addition to primary islands, while the 100–120 nm coating displayed tertiary islands (40 nm in diameter).⁴⁶ However, the trough-to-crest heights of these different nanopatterns were not significantly different. These studies suggest that these specific fractal patterns, and not the trough-to-crest heights, of the nanopatterns enhance osteoblastic differentiation and osteogenic potential. We propose that these optimized nanopattern geometries can be incorporated into 3D printed scaffolds by incorporating nanoparticles into the 3DP “ink.” This approach would create printed nanoscale geometries not currently attainable with traditional AM technologies.

We are developing a technique wherein piezo-electric (SonoPlot, SonoPlot, Inc., Middleton, WI) 3DP (Fig. 3) is used to print a suspension of calcium

Figure 3. Schematic of a 3DP approach to develop personalized bone scaffolds with a lip that matches the cross section geometry of the defect. **(a)** A micro-CT scan of the patient or animal model, combined with computer modeling and design, can be used to obtain the geometry of the defect that can be replicated during the bone graft fabrication. The personalized lip would provide additional support and fixation. **(b)** Illustration of the piezo-electric 3D printer. **(c)** Schematic showing the process of piezo-electric 3DP. The ultrasonic vibrations via the piezoelectric are used to deploy the colloidal suspension of calcium phosphosilicate particles of different diameters (20–100 nm) in polyvinylpyrrolidone.

Figure 4. Computer-generated image of the proposed personalized artificial bone scaffold with 3D nanopography. The balls are calcium phosphosilicate nanoparticles that can range in diameters of 10–100 nm. We envision that particles of only one given size would be used in a given construct. As the construct is resorbed, the nanopographic surface would remain constant.

phosphosilicate particles of different diameters (20–100 nm) in polyvinylpyrrolidone. This will result in a scaffold composed of calcium phosphosilicate particles of a desired nanometer diameter resulting in surface roughness on the nanoscale (Fig. 4). As the scaffold is resorbed, calcium phosphosilicate particles within the interior are exposed, assuring a constant nanopographic surface. Furthermore, using 3D medical imaging such as CT, combined with computer modeling and design, the geometry of the scaffold can be personalized to the geometry of the defect. One disadvantage of this approach is that it may not be amenable to the use of certain biomaterials that are difficult to 3DP.

A second approach that would be amenable to the use of several different biomaterials involves an innovative lost-mold rapid-infiltration forming (LM-RIF) process, developed at Penn State.⁴⁷ Using this technique, a mold, which could be personalized with imaging and computer modeling and design, is fabricated with a desired porosity and geometry. This mold could be cast with the calcium phosphosilicate suspension discussed above incorporating the desired optimized nanopography. Alternatively, it could be cast with other

biomaterials not amenable to 3DP. In this case, etching could be used to apply the desired nanopography on the surfaces of the scaffold. An advantage of a lost-mold approach is that, because the mold could potentially be fabricated using a wide variety of materials using mature AM processes, the attainable resolution of the mold (and subsequent casted scaffold) may be improved over the attainable resolution when directly printing the scaffold. Furthermore, casting may result in improved mechanical properties compared to direct printing. We have used LM-RIF to fabricate various objects (Fig. 5).⁴⁸

Figure 5. Detail of a gear fabricated using the lost-mold rapid-infiltration forming process. The gear was fabricated using tetragonal polycrystalline zirconia. It is possible to use a similar technique to fabricate artificial bone constructs or scaffolds.

In summary, 3DP and other AM approaches are promising technologies for developing artificial bone constructs or scaffolds that are equally as osteogenic as autografts or allografts. Furthermore, there is a strong potential to use medical imaging combined with computer modeling and design to develop artificial bone grafts personalized to an individual patient's particular defect.

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Author Disclosure Statement

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