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Review Article

3D-Printed Biopolymers for Tissue Engineering Application

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3D printing technology has recently gained substantial interest for potential applications in tissue engineering due to the ability of making a three-dimensional object of virtually any shape from a digital model. 3D-printed biopolymers, which combine the 3D printing technology and biopolymers, have shown great potential in tissue engineering applications and are receiving significant attention, which has resulted in the development of numerous research programs regarding the material systems which are available for 3D printing. This review focuses on recent advances in the development of biopolymer materials, including natural biopolymer-based materials and synthetic biopolymer-based materials prepared using 3D printing technology, and some future challenges and applications of this technology are discussed.

1. Introduction

Tissue engineering has been an area of immense research in recent years because of its vast potential in the repair or replacement of damaged tissues and organs [1, 2]. The present review will focus on scaffolds as they are one of the three most important factors, including seed cells, growth factors, and scaffolds in tissue engineering [3].

According to Hutmacher [4] a scaffold should satisfy the following criteria: (1) it should be bioresorbable and biocompatible with a controllable degradation and resorption rate to match cell/tissue growth *in vitro/vivo*; (2) it should have suitable surface chemistry for cell attachment, proliferation, and differentiation; (3) it should be three-dimensional and highly porous with an interconnected porous network for cell growth, flow transport of nutrients, and metabolic waste; (4) it should have proper mechanical properties to match the tissues at the site of implantation [5].

Random processes such as foaming [6], emulsification [7] solvent casting, particle/salt leaching [8, 9], freeze drying [10], thermally induced phase separation [11], and electrospinning [12, 13] have been used for the manufacturing of tissue engineering scaffolds. One major drawback is the fact that porous scaffolds fabricated by random processes cannot be produced with complete control of the geometrical parameters, such as pore size, pore interconnection size, and porosity.

Moreover scaffolds with tailored porosity for specific defects are difficult to manufacture with most of these approaches. Such scaffolds can be designed and fabricated using three-dimensional printing (3DP), which is becoming popular due to the ability to directly print porous scaffolds with designed shape, controlled chemistry, and interconnected porosity [14].

3D printing proposes an effective means to assemble all of these necessary components through the use of biomaterials, printing techniques, and even cell delivery methods.

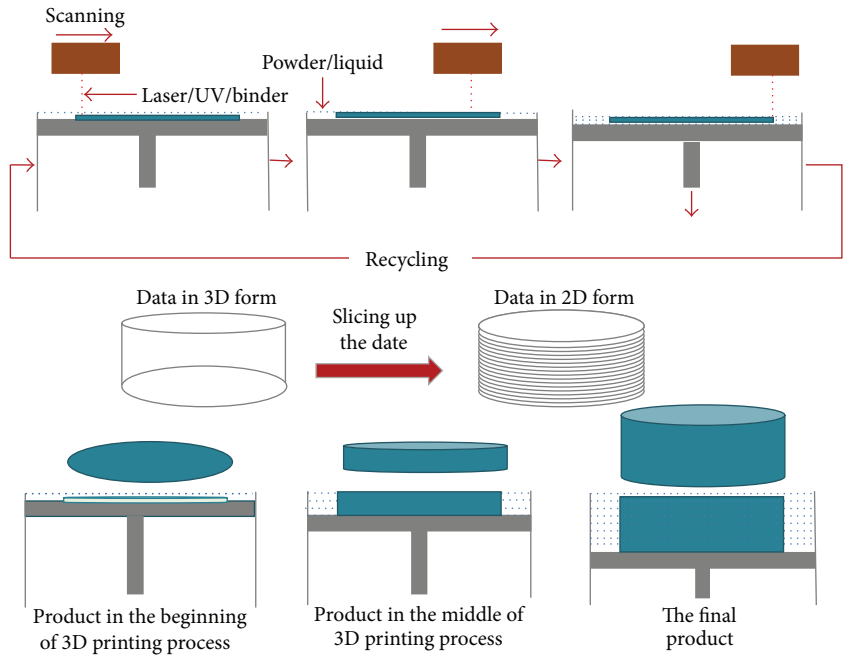


Figure 1: The principle schematic of 3D printing. The data that represents the products is sliced into two dimensions. The data, layer by layer, gets passed through the machine starting at the base of the product, and material is deposited layer by layer, infusing the newest layer of material to the old layer in an additive process.

The standard for 3D-printed tissue engineering constructs is to provide a biomimetic structural environment that facilitates tissue formation and promotes host tissue integration, including hard and soft tissues, whether through scaffold-based or scaffold-free approaches [15].

In this review, the commonly used biopolymers and basic principles of 3D printing will be introduced, as well as the development of polymers fabricated using 3D printing for tissue engineering. The development of natural polymer, synthetic polymer, and their composites is introduced, respectively, and the prospects of the 3D-printed biomaterials are discussed.

2. The Principle of 3D Printing Technology

3D printing is a process of making a three-dimensional object of virtually any shape from a digital model. 3D printing, which is additive manufacturing (AM) technology, is achieved using an additive process, where successive layers of material are laid down in different shapes. The 3D printing technology is used for both prototyping and distributed manufacturing, with applications in industrial design, automative industry, aerospace, architecture, medical industries, tissue engineering, and even food.

The first step of 3D printing is modeling. 3D printing takes virtual models from computer-aided design (CAD) or animation modeling software and "slices" them into digital cross-sections, so that the machine can successively use them as a guideline to print. Depending on the machine used, material or a binding material is deposited on the build bed or platform until the material/binder layering is complete and the final 3D model has been printed. The second step of 3D printing is printing. In this step, the machine reads the design from an stl file and lays down successive layers of liquid, powder, or some other materials to build the model from a series of cross-sections. At last, the 3D-printed object is finished according to the modeling. Some 3D printing techniques are capable of using multiple materials in the course of constructing parts and some may also utilize supports when building. Supports are removable or dissolvable upon completion of the print and the final object can be obtained. Postprocessing may be needed for some 3D-printed objects, while for others it may not be necessary. The principle schematic of 3D printing is shown in Figure 1.

A large number of additive processes including selective laser sintering (SLS), stereolithography (SLA), fused deposition modeling (FDM), and direct metal laser sintering (DMLS) are available for 3D printing. They differ among them in the materials that can be used and in the way the layers are deposited to create parts. For biopolymers, the most commonly used processes are SLS, SLA, and FDM.

In SLS, one of the many 3D printing technologies available, the slices are written by a carbon dioxide (CO_2) laser beam. The written process derives from the coalescence of particles through sinterization caused by the application of laser and thermal energy [16, 17]. The slice geometries are defined by selective sintering that follows the laser beam scanning onto the powder layers, according to the digital slices. The interaction of the laser beam with the powder raises the powder temperature to the point of melting and causes the particles to be fused together to form a solid mass.

The sintered material forms the object and the powder supports it during its manufacturing [18]. In the final stage of the SLS process the object is removed from the powder environment and it is cleaned from the remaining powder attached to its walls [17].

In SLA, thin successive layers are photocrosslinked by ultraviolet or visible light that induces photopolymerization of a reactive system according to a sliced CAD model. The technique requires a liquid photocrosslinkable resin with defined viscosity properties and, for that reason, reactive or nonreactive diluents are typically used. SLA can generate a large number of widely differing 3D structures in a reproducible way with precise control over the final microstructure and geometry [19]. In addition, nonlinear scaffold geometries can be produced.

In FDM, like other RP methods, one layer at a time is printed but typically the material is directly deposited on a surface where it is desired. Extrusion through a nozzle results in a cylindrical coiled morphology of each layer [20]. FDM uses a small temperature-controlled extruder to force out a thermoplastic filament material and deposit the semimolten polymer onto a platform in a layer by layer process. The monofilament is moved by two rollers and acts as a piston to drive the semimolten extrudate. At the end of each finished layer, the base platform is lowered and the next layer is deposited.

3. Materials Fabricated by 3D Printing for Tissue Engineering

In the search for alternatives to conventional treatment strategies for the repair or replacement of missing or malfunctioning human tissues and organs, promising solutions have been explored through tissue engineering approaches. Biomaterials-based scaffolds have played a pivotal role in this quest. At present, biomaterials have been widely used in skin [21, 22], cartilage [23], bone [24, 25], tendons [26], vessels [27, 28], nerves [29], bladder [30], and liver [31] tissue engineering. When designing a polymeric scaffold, a combination of biological and engineering requisites is considered within an application-specific manner. Material selection for tissue engineering applications is based on several important factors including biocompatibility, degradability, surface characteristics, processability, and mechanical properties [32].

Both natural and synthetic polymers have been used for biomedical applications. Natural polymers, such as collagen [33], chondroitin sulphate [34, 35], chitin [36, 37], and chitosan [38, 39], are widely used for tissue engineering and organ regeneration, since they facilitate cell attachment and maintenance of differentiation. Synthetic polymers such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly (glycolic acid) (PGA), poly(lactic-*co*-glycolic acid) (PLGA), and other synthetic polymers can provide extreme versatility regarding the control of their physicochemical properties and are generally easy to process into tissue engineering scaffolds [40–42]. Synthesis of these polymers can be tailored to yield a specific molecular weight, chemical structure, end group chemistry, and composition (homopolymers, copolymers, and polymer blends) in terms of tissue response. Furthermore, the biodegradation time of synthetic polymers makes them more attractive over natural ones [43].

Among the polymers used in tissue engineering, poly(α-hydroxy esters) (such as PLA, PGA, and PLGA) have attracted extensive attention for a variety of biomedical applications. Besides, PCL has been widely utilized as a tissue engineering scaffold. Blends and block copolymers of PCL with other poly(α-hydroxy esters) (such as poly(L-lactic acid-*co*-ε-caprolactone) (PLLACL) or poly(D,L-lactic acid-*co*-ε-caprolactone) (PDLLACL)) have been used to produce polymers with tailored properties [44–46]. In order to increase wettability, biocompatibility, or softness of bioresorbable polymers, blends and copolymers with nondegradable poly (ethylene glycol) (PEG) have been developed, such as block copolymers of PEG with poly(L-lactide) (PLLA), PLGA, and PCL [46, 47].

3.1. Natural Polymer and Its Composites Fabricated by 3D Printing Technology. Gelatin, the main component of hydrolyzed collagen, has been given much attention due to its natural origins in the extracellular matrix (ECM) and its ability to suspend cells in a gel at low temperatures [15]. Yan and others manually printed a liver tissue construct made of gelatin and chitosan mixed with hepatocytes followed by glutaraldehyde fixation [15, 48].

Research that explores natural polymers (such as starch) with water-based binders for use in direct 3D printing methods has shown promising results and can be combined with synthetic polymers for the desired biodegradable and mechanical properties. Starch-based polymers allow for increased degradation time and consequently expanded porosity as cellular integration increases, which is optimal for bone tissue engineering. A unique blend of starch-based polymer powders (cornstarch, dextran, and gelatin) was developed for the 3DP process by Lam et al. [49]. The scaffold properties were characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), porosity analysis, and compression tests. The analysis and tests demonstrated that porous 3D scaffolds created by a new blend of materials through 3DP were achievable. Starch and cellulose composites were shown to be biocompatible, which has been utilized in polymers designed for controlled drug delivery [50, 51] and bone cement [52, 53]. Salmoria et al. present the rapid fabrication of starch-cellulose and cellulose acetate scaffolds by SLS and the evaluation of the laser power, laser scan speed, and the polymer particle size influence on the scaffold properties [54]. The specimens with small particle sizes presented a higher degree of sintering and a significant level of closed pores, as indicated by the density measurements and mechanical tests, while the mechanical properties of specimens prepared with larger particles presented lower values of elastic modulus and tensile strength because of the low degree of sintering and limited number of unions. The results obtained showed that it is possible to fabricate biopolymer scaffold structures using starch-cellulose and cellulose acetate using SLS by process optimization based on the adjustment of laser power and scan speed. Specimens prepared with small particle size exhibit

satisfactory mechanical properties and level of porosity for the design and fabrication of scaffolds with potential use in tissue engineering and drug delivery [54].

The application of 3D printing in tissue engineering has enabled new methods for the printing of cells and matrix materials to fabricate tissue-analogous structures [55–57]. The practicality of using 3D printing to fabricate cell-laden constructs was demonstrated, where cells were localized as intended and the cell viability of the fabricated constructs was high.

Gelatin methacrylate was used to fabricate via the proposed projection stereolithography (PSL) platform, which can be used to design intricate 3D structure that can be engineered to mimic the microarchitecture of tissues, based on CAD [58]. Variation of the structure and prepolymer concentration enabled tailoring the mechanical properties of the scaffolds. A dynamic cell seeding method was utilized to improve the coverage of the scaffold throughout its thickness. The results demonstrated that the interconnectivity of pores allowed for uniform human umbilical vein endothelial cells (HUVECs) distribution and proliferation in the scaffolds, leading to high cell density and confluence at the end of the culture period. Moreover, immunohistochemistry results showed that cells seeded on the scaffold maintained their endothelial phenotype, demonstrating the biological functionality of the microfabricated GelMA scaffolds.

In 2014 Billiet et al. [59] reported the 3D printing of gelatin methacrylamide cell-laden tissue-engineered constructs with high cell viability for liver tissue engineering. They used VA-086 as a photoinitiator with enhanced biocompatibility compared to the conventional Irgacure 2959. A parametric study on the printing of gelatins was performed in order to characterize and compare construct architectures. The parameters including hydrogel building block concentration, the printing temperature, the printing pressure, the printing speed, and the cell density were analyzed in depth and optimized. The scaffolds can be designed having a 100% interconnected pore network in the gelatin concentration range of 10–20 w/v%. Control over the deposited strand dimensions can be guaranteed due to the physical properties of gelatin methacrylamide hydrogels and machine operating parameters. High viability (>97%) constructs displaying a maintained expression of liver specific functions were obtained using the VA-086 photoinitiator.

3.2. Synthetic Biopolymers and Their Composites Fabricated by 3D Printing

3.2.1. Synthetic Biopolymers Fabricated by 3D Printing. Among several tissues that are being actively researched, bone is one of the most widely studied, due to its critical functions in everyday life. When bone experiences disease or trauma, the defective portion often needs to be surgically removed [60]. Bone is able to self-regenerate; however, regeneration is limited to a distance of a few millimeters from healthy bone. Thereafter, a graft used to replace the removed portion of bone in order to restore functionality is required [61]. Autografting, which is the gold standard in filling bone defects, has known disadvantages, such as donor site morbidity, greater scar formation, and increased surgery times, as well as limited donor sites [62]. To enhance bone regeneration bone defects must be filled with an artificial porous spacer allowing the ingrowth of blood vessels and bone but restricting soft tissue ingrowth. In general, it is agreed that the porous network should consist of interconnected pores with a diameter in the range of 50–1000 μm [5]. Because of the obvious advantage of 3D printing, many researchers study the potential of 3D printing biopolymers for bone tissue engineering.

The most commonly used polymer for 3D porous scaffold is PCL, which, despite its good biocompatibility and processability, is rather hydrophobic leading to limited cell-scaffold interactions. Further, PCL is semicrystalline which together with its hydrophobicity and low water absorbing capacity, resulted in very slow degradation kinetics, which is regarded as a soft and hard tissue compatible bioresorbable material [43, 63]. Sudarmadji et al. chose PCL to fabricate 3D porous scaffolds using SLS [64]. Mathematical relations correlating scaffold porosity and compressive stiffness readings were formulated and compiled. In addition, cytotoxicity assessment was conducted to evaluate the toxicity of the fabricated PCL scaffolds [46]. The porosities, compressive stiffnesses, and yield strengths of the scaffolds varied in the ranges 40–84%, 2.74–55.95 MPa, and 0.17–5.03 MPa, respectively. This range of stiffness closely matches that of cancellous bone in the maxillofacial region. Besides, the chosen mode of fabrication for the PCL scaffolds has been proven to be feasible, as it is evident from the results of the cytotoxicity assessment. Elomaa et al. prepared and applied a photocrosslinkable PLC-based resin to build a porous scaffold using solvent-free SLA [65] (Figure 2). Photocrosslinkable macromers were prepared by methacrylating three-armed oligomers with methacrylic anhydride. Photocrosslinked networks had a high gel content, which indicates a high degree of crosslinking. Since macromers were heated above the melting temperature to obtain the suitable viscosity, no solvent was needed. No material shrinkage was observed after extraction and drying of the scaffolds due to the absence of solvent. The scaffolds accurately represented the structure modeled by computer-aided design. The average porosity was $70.5 \pm 0.8\%$, and the average pore size was 465 μm. The interconnectivity of the pores was high, indicating a great potential for these structures in cell seeding and implanting. PCL was developed as a filament modeling material to produce porous scaffolds, made of layers of directionally aligned microfilaments, using this computer-controlled extrusion and deposition process by Zein et al. in 2002 [66]. The PCL scaffolds were produced with a range of channel size 160–700 mm, filament diameter 260–370 mm, and porosity 48–77% and regular geometrical honeycomb pores, depending on the processing parameters. The scaffolds of different porosity also exhibited a pattern of compressive stress-strain behavior characteristics of porous solids under such loading. The compressive stiffness ranged from 4 to 77 MPa, yield strength from 0.4 to 3.6 MPa, and yield strain from 4% to 28%. Analysis of the measured data shows a high correlation between the scaffold porosity and

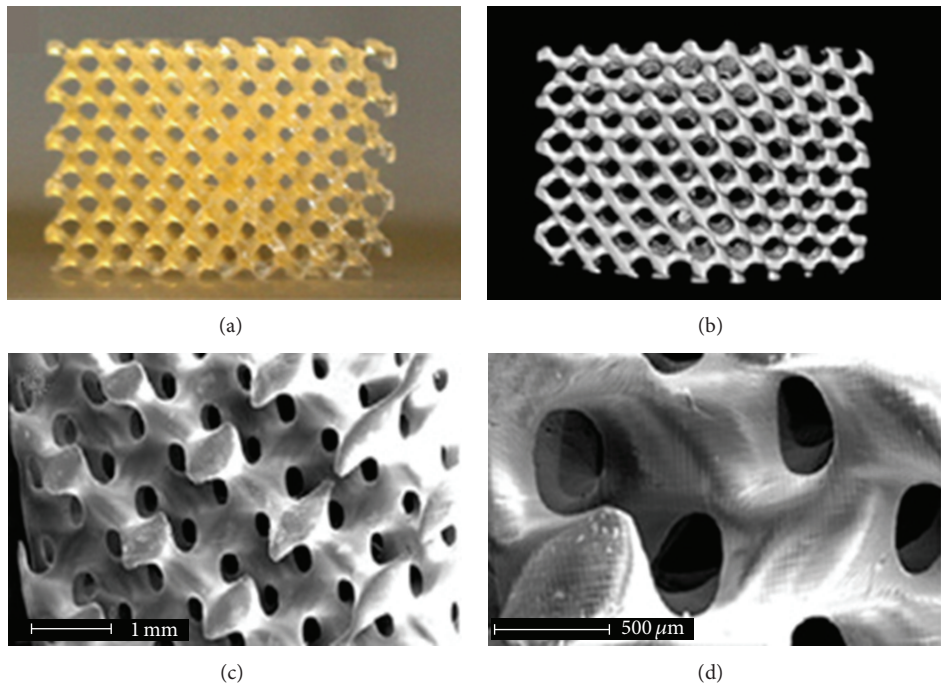


Figure 2: Photograph (a), μCT visualization (b), and SEM images ((c) and (d)) of a scaffold built by SLA using PCL macromere (the targeted theoretical molecular weights (Mn) of which was 1500 g/mol) [65].

the compressive properties based on a power-law relationship.

Cardiomyocytes are terminally differentiated cells and therefore unable to regenerate after infarction [67]. Myocardial infarction (MI) refers to necrosis or death of myocardial cells resulting from coronary occlusions. The medical condition called ventricular remodeling typically results in scar formation, heart wall thinning, and ventricular dilation [68, 69]. The major cause of progressive heart failure and death after myocardial infarction is the adverse remodeling [70] which has motivated the rapidly growing interest in developing regenerative therapies to restore the heart function and regenerate cardiomyocytes. Yeong et al. [69] present results on sintering PCL powder to fabricate a highly porous scaffold by SLS for application in cardiac tissue engineering. The tensile stiffness, mechanical property of sintered PCL struts, was characterized. C2C12 myoblasts were cultured on the scaffold in order to investigate the cellularity of the scaffold design for up to 21 days. Cell culture results were characterized using the MTS cell proliferation assay, F-actin and myosin heavy chain (MHC), and fluorescence immunostaining of the nucleus. Scaffolds produced by SLS with micropores are suitable for cell attachments and offer consistency and reproducibility in building complicated scaffolds. *In vitro* cell cultures using C2C12 cells showed the formation of multinucleated myotubes in the scaffold after 11 days of cell culture. A stable colony of cells was observed throughout 21 days of cell culture. This scaffold could potentially be used for cardiac and skeletal muscle tissue engineering.

There are some other polymers, in addition to PCL, that are used in 3D printing for tissue engineering. In 1998 Kim et al. [71] demonstrated the potential of 3DP technology when it is combined with salt leaching in the fabrication of polymeric scaffolds. The material used was copolymers of PLGA and a suitable solvent. They fabricated cylindrical scaffolds (8 mm in diameter and 7 mm in height) and managed to achieve interconnected porous channels of about 800 Am and microporosities of 45–150 Am by using salt leaching [49]. In 2001, scaffolds of varying pore sizes (38–150 Am) and void fractions (75% and 90%) were fabricated using PLLA and chloroform via the 3DP technology and salt leaching technique by Zeltinger et al. [72]. The cellular reactions to pore size and void fractions were investigated based on 3DP fabricated scaffolds. Cell proliferation was also observed on these scaffolds.

Poly(3-hydroxybutyrate) (PHB) is a natural thermoplastic polyester produced by microorganisms under imbalanced growth conditions and has attracted attention for applications in biomedical areas, such as in the production of scaffolds for tissue engineering due to its biocompatibility and biodegradability [17]. PHB processing by 3D printing can be made without the incorporation of additives such as plasticizers unlike the traditional methods. Oliveira et al. [73] successfully produced PHB porous structures with a height around 2.5 mm and pores with the size of 1 mm. Pereira et al. successfully produced PHB three-dimensional structures using 3D printing technology. The physical models showed dimensional features and geometries very close to the digital model. It was also demonstrated that the PHB powder was not altered after being submitted to 32.5 hours of SLS processing. The thermal properties of the physical model obtained with unprocessed PHB and PHB powder, which

underwent printing sets, did not show a significant difference between them. This corroborated the powder analysis, which indicated that the reuse of remaining material from a 32.15 hours-SLS did not affect the reproducibility of the process [17].

Recently, a functionalized aliphatic polyester based on PCL, namely, poly(hydroxymethylglycolide-*co*-ε-caprolactone) (PHMGCL) which possesses significantly higher hydrophilicity due to its hydroxyl groups attached to the backbone, resulting in a significant increase in human mesenchymal stem cell adhesion, proliferation, and differentiation as compared to PCL, was developed [74]. To evaluate the *in vivo* biodegradation and biocompatibility of 3D-printed scaffolds based on PHMGCL, which has enhanced hydrophilicity, increased degradation rate, and improved cell-material interactions as compared to its counterpart PCL, 3D scaffolds based on this polymer were prepared by means of fiber deposition (melt plotting) [75]. The biodegradation and tissue biocompatibility of PHMGCL and PCL scaffolds after subcutaneous implantation in Balb/c mice were investigated. The *in vitro* enzymatic degradation of these scaffolds was also investigated in lipase solutions. It was shown that PHMGCL 3D scaffolds lost more than 60% of their weight within 3 months of implantation while PCL scaffolds showed no weight loss in this time frame. The molecular weight (Mw) of PHMGCL decreased significantly after 3 months of implantation, while the molecular weight of PCL was unchanged in this period. *In vitro* enzymatic degradation showed that PHMGCL scaffolds were degraded within 50 h, while the degradation time for PCL scaffolds of similar structure was longer. A normal foreign body response to both scaffold types characterized by the presence of macrophages, lymphocytes, and fibrosis was observed with a more rapid onset in PHMGCL scaffolds. The extent of tissue-scaffold interactions as well as vascularization was shown to be higher for PHMGCL scaffolds compared to PCL ones. Therefore, the fast degradable PHMGCL which showed good biocompatibility is a promising biomaterial for bone and cartilage tissue engineering [75].

3.2.2. Synthetic Biopolymer-Based Composites Fabricated by 3D Printing. Griffith and others bound a mixed biodegradable polymer powder of 25% PLLA and 75% PLGA using chloroform as a binder to print a branched liver construct with internal architecture [76]. This design took host implantation into consideration by creating an artery- and vein-like inlet and outlet [15]. Organogenesis of liver tissue using 3D-printed PLLA/PLGA scaffolds has been investigated *in vitro* [14]. It was shown that culturing a mixture of hepatocytes and endothelial cells on a channeled biodegradable scaffold results in the desired tissue structure intrinsically.

Pati et al. [77] successfully printed 3D cell-laden constructs using the principle of hybrid structure fabrication, using PEG and PCL as materials. They printed an ear-shaped construct with a complex porous structure using the process described above. The use of sacrificial layer technology allowed the stacking of complicated structures regardless of geometrical shape. To fabricate the ear-shaped construct, a CAD model was generated from a computerized tomography (CT) scanned image of an ear; both the main framework and sacrificial parts were designed. A code generation process was then carried out via a motion program generator developed in house. In particular, two different hydrogels were printed into the framework, because native human ears consist of both cartilage and fat tissues.

Two photocrosslinkable hydrogel biopolymers, poly (ethylene glycol) dimethacrylate (PEG-DMA, MW 1000) and poly(ethylene glycol) diacrylate (PEG-DA, MW 3400), were used as the primary scaffold materials to prepare multimaterials [78]. Multimaterial scaffolds were fabricated by including controlled concentrations of fluorescently labeled dextran, fluorescently labeled bioactive PEG, or bioactive PEG in different regions of the scaffold. The presence of the fluorescent component in specific regions of the scaffold was analyzed with fluorescent microscopy, while human dermal fibroblast cells were seeded on top of the fabricated scaffolds with selective bioactivity and phase contrast microscopy images were used to show specific localization of cells in the regions patterned with bioactive PEG. Multimaterial spatial control was successfully demonstrated. In addition, the equilibrium swelling behavior of the two biopolymers after SL fabrication was determined and used to design constructs with the specified dimensions at the swollen state. The use of multimaterial SL and the relative ease of conjugating different bioactive ligands or growth factors to PEG allow for the fabrication of tailored three-dimensional constructs with specified spatially controlled bioactivity.

The majority of current protocols utilize water-insoluble photoinitiators that are incompatible with live cell-fabrication and ultraviolet (UV) light that is damaging to the cellular DNA. Various studies have reported the use of water-soluble dimethacrylatedpoly(ethyleneglycol)(PEG-DMA) to create structured, cell-containing hydrogels by stereolithography [79]. Dhariwala et al. were the first to successfully encapsulate (Chinese hamster ovary) cells in PEG-DMA hydrogels, using SLA [80]. Later, PEG-DMA constructs containing encapsulated human dermal fibroblasts [36] and PEG-diacrylate gel structures containing marrow stromal cells [81] were reported. In these cases, large numbers of cells could be capsulated at high densities (several millions of cells per mL) [82]. Lin et al. [79] reported the development of a visible light-based PSL system (VL-PSL), using lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as the initiator and polyethylene glycol diacrylate (PEGDA) as the monomer, to produce hydrogel scaffolds with specific shapes and internal architectures. Furthermore, live human adipose-derived stem cells (hADSCs) were suspended in PEGDA/LAP solution during the PSL process and were successfully incorporated within the fabricated hydrogel scaffolds. hADSCs in PEG scaffolds showed high viability (>90%) for up to 7 days after fabrication as revealed by live/dead staining. Scaffolds with porous internal architecture retained higher cell viability and activity than solid scaffolds, likely because of the increased oxygen and nutrients exchange into the interior of the scaffolds. The VLPSL can be applicable as an efficient and effective tissue engineering technology for point-of-care tissue repair in the clinic. Three-dimensional biodegradable PEG/PDLLA hydrogel structures

were prepared by Seck et al. using SLA at high resolutions [83]. The porous hydrogel structures had a well-defined pore network architecture, with a narrow pore size distribution and high interconnectivity of the pores. The resin and the built structures were compatible with cells. Upon seeding, human mesenchymal stem cells attached to the surfaces of the hydrogel structures and showed a spread morphology. After five days in culture, proliferation of the cells was observed. These hydrogel structures could therefore be used in tissue engineering, drug delivery, cell transplantation, and other biomedical applications.

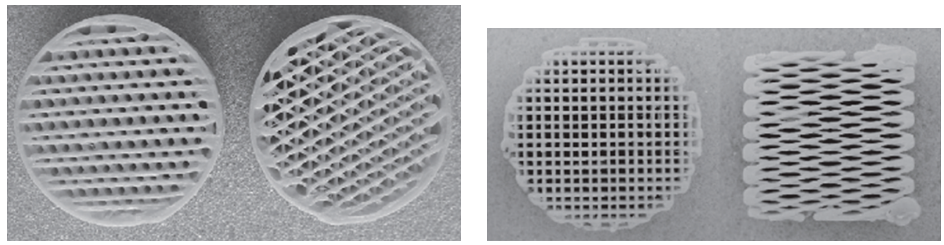
ABS is not widely used in medical devices in comparison to materials such as PCL and PLA which offer greater native biocompatibility [20]. Applications involving biological systems require materials that can minimize protein and any other biomolecule adhesion during flow. It is therefore of great interest to chemically modify the surface of ABS to engineer hydrophilicity and enable biocompatibility. Surface modification, specifically by the grafting of PEG, has long been shown to be a promising strategy to increase the biocompatibility of materials [20, 84]. McCullough and Yadavalli [20] examined the use of ABS as a core material for the construction of microdevices. A method to fabricate water-tight microfluidic devices using chemical dissolution via acetone is shown to render a porous FDM ABS device impervious to water flow between layers, while preserving the structural fidelity of printed microstructures down to 250 μm. A strategy is then presented that can enable the formation of a stable, biocompatible surface of ABS by the photoinduced grafting of PEG groups that improves the biocompatibility of the ABS by reducing the biofouling behavior. Surface characterization and protein adhesion studies are presented that demonstrate that this modified ABS represents a versatile material that can be used in fused deposition modeling to form microfluidic channels resistant to biofouling, thereby broadening the range of possible uses for ABS based FDM in microdevice and lab-on-a-chip type applications. The grafting caused the contact angle of the surface to be lowered from 77.58° for native ABS samples to below 40° for ABS-g-PEG and reduced the adhesion of the protein BSA. The results clearly indicate that sealing an FDM ABS surface with an acetone treatment can be achieved with minimal effect on the device and the grafting of PEGMA onto ABS is a viable method to increase surface hydrophilicity and biocompatibility.

Currently, composites of polymers and bioactive inorganic materials are being developed with the aim of increasing the mechanical scaffold stability and improving tissue interaction. By combining biodegradable polymers and bioactive ceramics, such as hydroxyapatite (HA) and tricalcium phosphate (TCP), calcium phosphate (CaP) composite scaffolds were made by 3D printing. Nanosized osteoconductive calcium phosphates (Ca-Ps), including HA, tricalcium phosphate (TCP), and substituted HA and TCP, have gained much recognition in biomaterials development due to their small size, high surface area to volume ratio, and biomimetic similarity to natural bone structure when combined with biopolymers such as collagen, PLLA, and chitosan [17, 85, 86].

Hydroxyapatite ($Ca_{10}(PO4)_6(OH)_2$) has attracted much interest due to its chemical similarity to the calcium phosphate mineral present in biological hard tissues [87, 88]. HA has been used for a variety of biomedical applications, such as a matrix for controlled drug release and a carrier material in bone tissue engineering [89]. Recently, nanosized hydroxyapatite (nHA) has been highlighted due to its advantageous features over conventional microsized materials. nHA has the potential to act as a carrier of therapeutic agents, enabling controlled drug release extracellularly or intracellularly, and at the same time it has high absorbability in the body for the regeneration of hard tissue [90, 91].

PCL/HA composite has attracted a great interest for the bone tissue engineering application. Wiria et al. researched the use of biocomposite materials, consisting of PCL and HA, to fabricate tissue engineering scaffolds via the SLS technology in 2007 [92]. Simulated body fluid (SBF) samples show the formation of hydroxy carbonate apatite, as a result of soaking HA in a SBF environment. Cell culture experiments showed that Saos-2 cells were able to live and replicate on the fabricated scaffolds. The results show the favorable potential of PCL/HA biocomposites as tissue engineering scaffolds that are fabricated via SLS. Eosoly et al. [93] studied PCL/HA composite scaffolds in the same year. In their study HA and PCL, which were considered suitable for hard tissue engineering purposes, were used in a weight ratio of 30 : 70. Four parameters, namely, laser fill power, outline laser power, scan spacing, and part orientation, were investigated according to a central composite design. A model of the effects of these parameters on the accuracy and mechanical properties of the fabricated parts was developed. The compressive modulus and yield strength of the fabricated microstructures with a designed relative density of 0.33 varied between 0.6 and 2.3, 0.1 and 0.6 MPa, respectively. The mechanical behavior was strongly dependent on the manufacturing direction. Eshraghi and Das [94] studied the experimental characterization of the compressive mechanical properties of PCL-HA composite scaffolds prepared by SLS technology for bone tissue engineering. In their study, they further establish the ability of SLS to manufacture PCL-HA composite scaffolds with near-full density in designed solid regions for bone tissue engineering. The mechanical properties of the PCL-HA composites showed improvement over that of pure PCL. They also demonstrate that the mechanical properties of these scaffolds can be predicted before manufacturing with high accuracy. A direct extension of being able to predict the mechanical properties of composite materials at any filler loading in combination with a direct fabrication method with the capability to produce complex anatomic parts is the ability to custom design both the material properties and the anatomical shape of tissue-engineered constructs for both patient and site-specific recovery strategies.

A poly(D,L-lactide)/nanosized hydroxyapatite (PDLLA/nHA) composite resin was prepared and used to fabricate composite films and computer designed porous scaffolds by Ronca et al. [95] using SLA, mixing varying quantities of nHA powder and a liquid photoinitiator into a photocrosslinkable PDLLA-diacrylate resin. The influence of nHA on the rheological and photochemical properties of the resins was



(a) (b)

Figure 3: Three dimensionally interconnected controlled porosity PP-TCP composite scaffolds with different internal architecture using FDM process [101].

investigated, with the materials being characterized with respect to their mechanical, thermal, and morphological properties after postpreparation curing. In the cured composites stiffness was observed to increase with increasing concentration of nanoparticles. With increasing ceramic component the resins became more viscous, and NMP was added as a nonreactive diluent to decrease the viscosity and allow processing by stereolithography. SEM images showed exposed ceramic particles on the pore surface, allowing interaction between the bone-forming nHA and cells.

Calcium phosphate ceramics have the ability to induce osteogenic differentiation of human adipose-derived stem cells by osteoinduction [96–99]. Three-dimensional na-nocomposite scaffolds based on calcium phosphate (Ca-P)/poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) and carbonated hydroxyapatite (CHAp)/PLLA nanocomposite microspheres were successfully fabricated by Duan et al. [100] using SLS. The optimized scaffolds had controlled material microstructure, totally interconnected porous structure, and high porosity. The morphology and mechanical properties of Ca-P/PHBV and CHAp/PLLA nanocomposite scaffolds as well as PHBV and PLLA polymer scaffolds were studied. Biological evaluation showed that SaOS-2 cells had high cell viability and normal morphology and phenotype after 3- and 7-day culture on all scaffolds. The incorporation of Ca-P nanoparticles significantly improved cell proliferation and alkaline phosphatase activity for Ca-P/PHBV scaffolds, whereas CHAp/PLLA nanocomposite scaffolds exhibited a similar level of cell response compared with PLLA polymer scaffolds. The three-dimensional nanocomposite scaffolds provide a biomimetic environment for osteoblastic cell attachment, proliferation, and differentiation and have great potential for bone tissue engineering applications. Particulate-reinforced polymer-ceramic composites were developed by high shear mixing of polypropylene (PP) polymer and tricalcium phosphate (TCP) ceramic [101] (Figure 3). Processing aids were used to improve plasticity and processability to the composites. Controlled porosity scaffolds were fabricated via the FDM. These porous scaffolds were characterized for their use as bone grafts in terms of physical, mechanical, and biological properties. Hg porosimetry was performed to determine pore size and their distribution. Scaffolds with different complex internal architectures were also fabricated using this composite material. Tensile properties of neat PP, PP with processing aids (without TCP), and PP-TCP composite (with processing aids) were evaluated and compared using standard dog bone samples. Uniaxial compression tests were performed on cylindrical porous samples with an average pore size of 160 Am and varying vol.% porosity (36%, 48%, and 52%). Samples with 36 vol.% porosity showed the best compressive strength of 12.7 MPa. Cytotoxicity and cell proliferation studies were conducted with a modified human osteoblast cell line (HOB). Results showed that these samples were nontoxic with excellent cell growth during the first two weeks of *in vitro* testing.

Bioactive glass is known to benefit cell interactions of polymeric tissue engineering scaffolds [102, 103]. Most likely, the best response is obtained when the glass is on the scaffold surface without a cover. Miller and others recently developed a 3D fiber drawing system to fabricate perfusable carbohydrate glass lattices coated with a thin layer of poly(d-lactide-*co*-glycolide), resembling patterned vascular networks [104]. Elomaa et al. [105] combined a photocrosslinkable PCL resin with bioactive glass and fabricated the composite scaffold. Bioactive glass was homogeneously distributed through the highly porous scaffolds and their surface. The presence of calcium phosphate deposits on the surface of the composite scaffolds indicated *in vitro* bioactivity. The bioactive glass increased the metabolic activity of fibroblasts. The research showed that SLA technology enables the fabrication of well-defined composite scaffolds in which the bioactive glass is homogeneously distributed on the surface and readily available for rapid ion release and cell interactions. By SLA, an unwanted polymer layer covering the BG particles on the scaffold surface can be successfully avoided. The study suggested that photocrosslinked composite scaffolds of BG and PCL prepared by SLA technology had great potential as bioactive and biodegradable supports for cells in regenerative medicine.

Serra et al. combined PEG and CaP glass particles with the PLA matrix to fabricate 3D biodegradable porous composite scaffolds [106]. The 3D printing technique permitted the fabrication of highly porous scaffolds with mechanical properties considerably higher than other methods commonly used to fabricate 3D polymer scaffolds. The addition of the soluble CaP glass particles (and PEG) to the PLA matrix changed both the morphology and the physicochemical properties of the surface of the materials which affected

cell behavior. Surface properties were also assessed, showing that the incorporation of glass particles increased both the roughness and the hydrophilicity of the scaffolds. Mechanical tests indicated that compression strength is dependent on the scaffold geometry and the presence of glass. Preliminary cell response was studied with primary mesenchymal stem cells (MSC) and revealed that CaP glass improved cell adhesion. Overall, the results showed the suitability of the technique/materials combination to develop 3D porous scaffolds and their initial biocompatibility, with both being valuable characteristics for tissue engineering applications.

4. Prospects and Conclusions

By the year of 2014, 3D printing technology has been studied by biotechnology firms and academia for possible use in tissue engineering applications in which organs and body parts are built using inkjet techniques. One of the main advantages of 3D printing is that it allows the manufacturing of objects having complex geometries and intricate internal structure, which can be designed according to the needs of individual patients using their 3D medical scan data. A great prospect for biomedical applications, especially for tissue engineering applications, has been shown. Both natural and synthetic polymers have been developed for tissue engineering via the 3D printing technology, and numerous additional materials are being developed. Fibers and particles have been combined with polymers to fabricate materials with better bioactivity and biocompatibility, as well as physical and chemical properties.

For a wider application of 3D printing, the moral problem related to the 3D-printed organ for medical application should be further studied, the cost of 3D printers should be less, and more materials systems which are available for 3D printing should be developed. A further study on the mechanism of cell attachment, differentiation, and growth within the 3D-printed materials should be carried on as well.

With the development of nanotechnology, materials such as nanotubes [107–110], nano-structured particles [111], nanofibers [112, 113], and other nanosized materials [114–116] have been fabricated. Nanomaterials have special mechanical, electrical, magnetic, optical, chemical, and other biological properties due to their high aspect ratio and surface area [117–119]. Many nanomaterial surfaces exemplify high (bio- and cyto-) compatibility, by promoting protein adsorption and enhancing subsequent cellular adhesion and tissue growth more than on conventional flat implant surfaces such as titanium, ceramics, and biopolymers [114]. Nanocomposites attract more attention because of their potential combination of properties from both the nanomaterials and the host materials matrix [108, 120–122]. It is promising to incorporate nanomaterials in 3D printing. The nanomaterials can be introduced into 3D printing in the following way: (1) premixing the nanomaterials into the host matrix before 3D printing and (2) introducing the nanomaterials at the intermittent stoppages of the 3D-printed host matrix [123]. All in all, 3D printing will help to expand the application of nanomaterials for tissue engineering.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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