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Extrusion-based 3D printing technologies for 3D scaffold engineering

10

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10.1 Introduction

Additive manufacturing (AM) technologies (a.k.a. 3D printing), have been extensively used for fabrication of synthetic tissue engineering (TE) scaffolds. AM technologies are automated techniques that can simply fabricate 3D structures with controlled internal and external geometry layer-by-layer using computer-aided design (CAD) file. Initially, these technologies were used for product design and development and rapid prototyping due to their significant reduction in lead time and cost, while in recent years their applications have been expanded to high-value production and specialized manufacturing. A large number of AM processes have been developed allowing the use of various materials ranging from plastics and metals to advanced ceramics and composites for various industries [1]. The AM technologies play an increasingly important role in the biomedical industry so that medical and dental has established itself as a strong sector for AM. The AM techniques have been used within medical/dental area for the production of assistive surgical and prosthetic devices, customized surgical implants, dental implants, drug delivery devices, and more importantly, TE scaffolds [1].

The use of these technologies for the manufacture of TE scaffolds is widely explored as they can overcome limitations of conventional porous material manufacturing methods (such as solvent casting/salt leaching, and phase separation) in terms of geometry and process consistency [2]. In addition, 3D printed scaffolds have shown considerable improvement in terms of biological performance. According to a recent report by Sun and colleagues [3], the metabolism of cells seeded on the 3D printed silk fibroin/collagen (SF/C) scaffolds was more viable than the metabolism on the scaffolds prepared by vacuum freeze-dried technique. Also, the number of cells in the 3D printed scaffold was higher in comparison to a similar measurement on the freeze-dried material. Consequently, stem cells grew well inside the 3D printed scaffolds, while the internal structure of the freeze-dried scaffold was disordered. So, compared with the freeze-dried technique (as a

traditional technique), the 3D printed scaffold exhibited better overall performance and was more suitable for cartilage tissue engineering [3].

p0020 The application of the material extrusion AM systems comprising a movable extrusion head controlled by computer has spawned a range of construction methods. In these processes, continuous flow of materials in the form of paste or thick ink is dispensed layer-by-layer using the extrusion nozzle(s) incorporated with a 3D motion system. Much attention has been paid to material extrusion AM systems in tissue engineering in recent years as they are mechanically simple in comparison to other AM techniques and a wide range of biomaterials can be processed effectively. The additive nature of the extrusion-based AM processes ensures minimal waste of biomaterial and makes it suitable for mass production of scaffolds, multiple material porous bioactive structures [4], and micro-scale woodpile structures [5]. Extrusion-based AM systems have been developed specifically for the processing of biological materials which are called “bio-printers.” The bio-printers can deposit various biopolymers, hydrogels, and encapsulated multiple cell types in hydrogels to produce 3D bioconstructs with unique biological features [6–8].

p0025 Basically, the extrusion AM systems are classified into two main sub-groups: processes based on material melting, and processes without material melting [9]. Fused deposition modeling (FDM), precision extrusion deposition (PED) [10], 3D fiber deposition [11], precise extrusion manufacturing (PEM) [12], and multiphase jet solidification (MJS) [13] are the main AM techniques based on the melting process. Pressure-assisted microsyringe (PAM) [14], low-temperature deposition manufacturing (LDM) [15], 3D bioplotting [16], robocasting [17], direct-write assembly [18], and solvent-based extrusion freeforming (SEF) [19] are the most commonly used AM techniques without material melting. Four major nozzle designs have been exploited in non-heating processes: pressure-actuated, volume-driven injection nozzles (normally using a stepper-motor), solenoid, and piezoelectric-actuated (whereas two main nozzle designs, namely, filament driving wheels and mini-screw extruder have been used in processes with material melting [4]). Different extrusion-based 3D printing techniques and their applications are reviewed in this chapter and the SEF method is addressed with in-depth discussion about its recent advances and applications.

s0015 **10.2 Extrusion-based AM systems with material melting**

p0030 The FDM process is the first extrusion AM system developed based on polymer melts extrusion for 3D printing of high strength functional parts. Thermoplastic materials in the form of filament are used as feedstock, and a pinch roller or screw-feed mechanism is used to push the filament into a liquefier, and subsequently extrude from a computer-controlled nozzle. A variety of modified FDM systems has been developed for fabrication of biomaterials 3D scaffolds with micron-size pores and struts. Xiong et al. [12] developed the PEM process in which compressed air is used instead of a piston or rotating screw to expel the melted biomaterial through the deposition nozzle.

- p0035 Hutmacher's group [20] has extensively investigated the process parameter for printing polycaprolactone (PCL)-based composite scaffolds. They printed various composites including PCL, PCL/hydroxyapatite (HA), and PCL/tricalcium phosphate (TCP) scaffolds with resolution of 250 μm using FDM process. Bone bio-composite scaffolds produced from polymer and calcium phosphates (such as HA and TCP) using FDM process have good mechanical and degradation properties, improved cell seeding, and enhanced incorporation and immobilization of growth factors [20]. Kalita et al. [21] produced controlled porosity, polymer-ceramic composite polypropylene (PP)/TCP scaffolds, with 3D interconnectivity designed to promote a richer supply of blood, oxygen, and nutrients for healthy in-growth of bone cells. Controlled porosity alumina and β -TCP ceramic scaffolds with pore sizes in the range of 300–500 μm and pore volumes of 25%–45% have also been produced using the indirect FDM process [22]. Safari's group [23] produced hybrid scaffold from alumina and wax (as support structure) directly using multi-nozzle fused deposition of ceramics (FDC). In 2004, Woodfield et al. [11] developed the 3D fiber deposition process with the aim of extrusion of highly viscous polymers.
- p0040 The PED process is another adjuration of melt extrusion AM process developed by Wei Sun's group [10] in which biomaterial in the form of pellet or granule is liquefied in a chamber and a rotating screw (mini-extruder) expels the molten biomaterial through a nozzle. The Polytechnic Institute of Leiria developed a variation of FDM called "BioExtruder" [24,25] for printing PCL scaffolds. Later the technique was used for printing PCL/HA composites [26]. Highly porous poly(lactic-co-glycolic acid) (PLGA) scaffolds for cartilage tissue engineering were fabricated by Hung-Jen et al. [27] using an FDM process and were further modified by type II collagen. Recently, Poly(lactic acid) (PLA)/15 wt% HA porous scaffolds with a pre-modeled structure were obtained using a fused filament fabrication method [28]. Tellis et al. [29] used micro computed tomography (CT) to create biomimetic polybutylene terephthalate (PBT) trabecular scaffolds.
- p0045 Although there have been a large number of publications in the field of extrusion-based 3D printing with material melting, so far there have been very few reports on extrusion 3D printing of high temperature biopolymers such as polyether-ether-ketone (PEEK). PEEK has excellent cell biocompatibility and mechanical properties such as strength and elastic modulus comparable to cortical bone [30,31]. Medical grade PEEK-OPTIMA has been developed to meet the FDA's requirements and has been used in multiple clinical applications including spinal cage fusion and crani-omaxillofacial reconstruction [32,33]. It is quite challenging to process PEEK through extrusion freeforming due to its very high melting temperature in comparison with other biopolymers such as PLA. The preliminary report on extrusion AM of PEEK is promising [34]. However, very small parts could be printed with insufficient quality (e.g., defects such as part warpage and/or delamination) and no further discussion of the main challenges and obstacles, and mechanical properties has been presented. The first comprehensive report on successful extrusion AM of medical grade PEEK structures with in-depth discussion of the main challenges was presented by Vaezi et al. [35]. Fig. 10.1 depicts some

Figure 10.1 (A) 3D printed PEEK spinal fusion cage with 400 μm pores to enhance osseointegration; (B) large size PEEK samples 3D printed in 0.2 mm layer thickness. *Source:* Taken from M. Vaezi, S. Yang, Extrusion-based additive manufacturing of PEEK for biomedical applications, Virtual Phys. Prototyp. 10 (2015) 123–135.

extrusion-based 3D printed PEEK parts including a new porous spinal fusion cage design for improvement of osseointegration (Fig. 10.1A).

Different manufacturing techniques such as injection molding, particulate leaching, compression molding, and SLS have already been used to produce porous PEEK for biomedical applications [36–38]. Conventional techniques such as particulate leaching have poor control on porosity, and suffer from limitations such as inconsistency and manual intervention. The SLS process has made a great breakthrough in PEEK implant design and manufacturing due to its capability to 3D print directly using CAD file with a high level of repeatability. However, it is a high cost AM process which suffers from limitations such as achievable pore size and very low recycle rate of PEEK powder. In contrast, extrusion-based 3D printing, as a low-cost AM method, offers a great control on macro/microarchitecture, and guarantees reproducibility. The use of extrusion freeforming permitted excellent control on pore size and interconnectivity in the printed PEEK samples which is necessary for bone ingrowth [35]. In addition, the porous PEEK implants can be rapidly printed with a much higher control level on external shape and porosity with enhanced reproducibility, a key requirement in the production of medical devices.

10.3 Extrusion-based AM systems without material melting

Two major limitations of FDM process are the need to use filamentary materials as feedstock and the high heat effect on the raw biomaterial which can result in

degradation of the biomaterial. To overcome these problems, variety of extrusion 3D printing systems without materials melting were proposed. PAM process is a technique developed by Vozzi and co-workers [14] without the need for heating. PAM uses a pneumatic-driven microsyringe to deposit biomaterial on a substrate. High resolution polymeric scaffolds with various compositions such as PCL, poly-L-lactic acid (PLLA), PLGA, and PCL/PLLA, gelatin, and alginate hydrogels scaffolds with three different geometries-square grids, hexagonal grids, and octagonal grids could be printed using PAM method [39–41].

p0060 LDM process is another low-temperature extrusion AM method proposed by Xiong et al. [12] that its key feature is a non-heating liquefying processing of materials. In this process, material slurries are fed into the material supply that is connected to a screw pump nozzle using a soft pipe and fabrication process is accomplished in low temperature environment under 0°C in the refrigerator. The layer of deposited materials is frozen on the platform. After the forming process, the frozen scaffolds need to be freeze-dried for a rather long time (~38 hours) to remove the solvent. Recently, a new 3D printing system based on LDM was developed for the fabrication of conductive 3D nanocomposite-based microstructures with arbitrary shapes. This technology consists in the additive multilayer deposition of polymeric nanocomposite liquid dispersions based on PLA and multi-walled carbon nanotubes (MWCNTs) by means of extrusion-based 3D printer [42].

p0065 Ang et al. [43] set up a special robotic extrusion device called “rapid prototyping robot dispensing (RPBOD)” for the design and fabrication of chitosan-HA scaffolds. The RPBOD system consists of a computer-guided desktop robot and a one-component pneumatic dispenser. A mixture of sodium hydroxide solution and ethanol with different ratios was used as plotting medium to produce chitosan-HA scaffolds.

p0070 3D bioplotting is another technique that was first developed by Landers and Mulhaupt [16] to produce scaffolds for soft tissue engineering purposes, and simplifying hydrogel manufacturing. In this process, the material dispensing head normally moves in three dimensions, while the fabrication platform is stationary. Either a filtered air pressure (pneumatic nozzle) or a stepper-motor (volume-driven injection nozzle) is used to plot a viscous material into a liquid (aqueous) plotting medium with a matching density. The work by Landers and Mulhaupt led to the commercialization of the first 3D-Bioplotter by EnvisionTec GmbH (www.entiontec.com) to fulfill the demand for 3D scaffolds with well-defined external and internal structures in tissue engineering and controlled drug release. Recently, water-based 3D printing materials with controlled bioactivity for customized cartilage tissue engineering was developed to be processed through bioplotting method. The printing ink contains the water dispersion of synthetic biodegradable polyurethane (PU) elastic nanoparticles, hyaluronan, and bioactive ingredients TGFβ3 or a small molecule drug Y27632 to replace TGFβ3 [44].

p0075 Different extrusion AM techniques have also been developed for printing hard bioceramic tissue engineering scaffolds. Robocasting is a ceramic processing technique in which a computer controls the robotic deposition of highly concentrated (typically 50–65 vol% ceramic powder) colloidal ceramic slurries [17]. The slurry is deposited layer-by-layer from a syringe using constant displacement at a controlled rate. Miranda et al. [45,46] used robocasting process to produce β-TCP

scaffolds with designed 3D architecture and mesoscale porosity using concentrated inks with suitable viscoelastic properties. The deposition was done in a non-wetting oil bath to prevent non-uniform drying during printing. Direct-write assembly is the next generation of robocasting method developed by Lewis et al. [18] whereby a wider range of inks can be printed in both planar and 3D structures. Robocasting and direct-write assembly are essentially identical—the primary difference is the way in which ink is extruded. Robocasting relies on a constant displacement process, whereas direct ink writing relies on a constant pressure process. Using bio-compatible inks, Lewis et al. printed 3D scaffolds and microvascular networks for tissue engineering and cell culture [47]. Different 3D HA scaffolds [48] and scaffolds composed of a gradient array of silk/HA were fabricated by direct-write assembly [49]. The 3D silk/HA scaffolds are used to support the growth of co-cultures of human bone marrow derived from mesenchymal stem cells (hMSCs) and human mammary microvascular endothelial cells (hMMECs) to assess in vitro formation of bone-like tissue. In addition, 3D microperiodic scaffolds of regenerated silk fibroin have been fabricated using direct-write assembly for tissue engineering [50]. Later, biocompatible silk optical waveguides as fine as 5 μm were printed by direct-write assembly of a concentrated silk fibroin ink through a micro-nozzle into a methanol-rich coagulation reservoir [51]. High resolution 3D micro-periodic hydrogel scaffolds with 1 μm filaments were also printed for guided cell growth by direct writing of a poly(2-hydroxyethyl methacrylate) (pHEMA)-based ink through a gold-coated deposition micronozzle that is simultaneously photopolymerized via UV illumination [52]. In a more recent work, concentrated alginate/polyvinyl alcohol (PVA) bio-ink was prepared for 3D bioprinting [53]. Proteins and growth factors were co-printed into scaffolds and they achieved a controlled release from scaffolds. Direct-write assembly has also been used for printing hard bioceramic structures. The compressive strength of direct-write assembled hard zirconium dioxide (ZrO_2) scaffolds with porosity about 63% was reported in the range of 8 MPa [54]. A novel type of macroporous ceramic scaffolds comprising hollow tubular filaments with a highly microporous structure could be printed using 3D ceramic/camphene-based co-extrusion (3D-CoEx) method [55].

p0080 SEF is another extrusion AM technique developed by Evans's and Yang's group [56–61] to produce ceramic scaffolds. SEF is a relatively simple process in which phase change is based on solvent evaporation. Paste with high yield strength is prepared by blending polymer, ceramic, and a solvent with specific ratios. This process involved the following steps: (1) preparation of bioceramic paste; (2) 3D printing; and (3) drying, debinding, and sintering of the 3D printed scaffold. A range of bioceramic scaffolds have been fabricated using the SEF method with various compositions (different HA/ β -TCP ratios) and sintered from 1100 to 1300°C [57]. In addition to calcium phosphates, bioceramic pastes such as alumina, alumina/silica, zirconia, and alumina/graphite have also been used through the SEF method for fabrication of 3D lattice structures with fine filaments [61,62].

p0085 Although scaffolds 3D printed from bioceramics have shown promising results for bone repair, their application in repairing load-bearing long bone is limited due to their poor mechanical properties in comparison to human bone. To overcome this problem, freeform extrusion fabrication of bioactive silicate 13–93 glass

scaffolds reinforced with titanium (Ti) fibers was investigated [63]. In another work, Mg-doping wollastonite (CSi-Mg)/b-TCP scaffolds were developed with high strength via extrusion 3D printing technology [64]. Recently, a simple extrusion-based 3D printing technique was developed by Srivas and co-workers [65] to produce porous Ti6Al4V scaffold under ambient environmental condition. 3D printed Ti6Al4V scaffold with pore size $\sim 500 \mu\text{m}$ and total porosity $\sim 58\%$ was achieved. The scaffold exhibited $\sim 13\%$ shrinkage after sintering.

p0090 As a consequence of what was discussed above, extrusion-based AM processes can be employed as a standard tool in biofabrication of tissue engineering scaffolds. Scaffolds-based tissue engineering is a proven approach in regenerative medicine but is still subjected to some limitations and challenges including: (1) complications posed by host acceptance (immunogenicity, inflammatory response, mechanical mismatch), and; (2) problems related to cell cultures (cell density, multiple cell types, specific localization) [66]. Cell-based printing techniques have been intensively investigated and many innovative approaches such as organ bioprinting [67], laser writing of cells [68], and biological laser printing (BioLP) [69] have been introduced to complement limitations in scaffold-based tissue engineering. Several extrusion-based systems such as 3D bioplotter described earlier can be served as a bio-printer, if sterile conditions can be acquired. The scaffold-free cell printing technologies has opened up new possibilities, however, an ideal 3D tissue or organ has not yet been printed successfully since gel-state cell-embedded hydrogels are unable to maintain the desired 3D structure due to their insufficient mechanical strength [4]. Recently, a hybrid cell-printing technique that combines a conventional extrusion-based cell-printing process with an electrohydrodynamic jet was proposed to overcome this problem [70]. The electric field stabilizes the extruded struts of cell-embedding-hydrogel and reduces the damage to dispensed cells caused by the high wall shear stress in the dispensing nozzle. Hybrid scaffolds were also developed to increase mechanical strength of the printed bioconstructs [71]. A hybrid biopolymer/cell bioconstruct printed by Shim et al. [72] using multi-head tissue/organ building system (MtoBS). Two different alginate solutions were infused into the previously prepared PCL framework to create the 3D construct for osteochondral printing. The PCL struts provide controllable mechanical support of the cell laden alginate struts which provide biological activity. Using the hybrid method, the subsequent mechanical properties of the scaffolds could fundamentally enhanced and could be engineered as those of local tissues. Besides, since the hydrogel is bolstered by the thermoplastic material, a more choice of hydrogels and concentrations can be utilized in comparison with scaffold-free method, which can result in enhancing the conditions for encapsulated cells to proliferate and deposit new matrix [73].

s0025 **10.4 Production of bioactive composites using the SEF process**

p0095 The key strength of the SEF method is its low cost, repeatability, and productivity. Basically, the fabrication speed and cost is very important for tissue engineering

Figure 10.2 (A) Rapid manufactured bioceramic scaffolds with a high level of consistency using SEF method; (B) close view of the PLA/HA (40 vol% HA) biocomposite scaffold with HA particles on the surface for cell attachment; (C) a typical porous biocomposite tube produced by rolling SEF printed flexible PIA/HA.

applications as normally a series of samples needs to be prepared with a high level of consistency for further in vitro and in vivo tests. Using the SEF method, a large number of TE scaffolds can be produced at low cost and very quickly with a high level of consistency. In fact, the SEF method is considered to be a true rapid manufacturing technique enabling tissue engineers to produce scaffolds with desired macro/microstructure in a short period of time at low cost. The versatility of the SEF method is not just in terms of process cost, speed, and consistency but its capability to process various high-demanding ceramics (such as zirconia, alumina, HA, β -TCP, Bioglass, etc.) to serve different applications. Fig. 10.2A depicts rapid manufactured HA, alumina, and zirconia scaffolds prepared in a short period of time (each at <15 minutes including ceramic paste preparation time).

Through control of the printing parameters such as solvent content in the paste, nozzle size, paste deposition speed, and build layer thickness it was possible to determine the microstructure of the scaffolds. The 3D printed scaffolds have significant uniform filament and pore size which proves the ability of the process to accurately control microstructure. The quality of the high resolution lattices produced depended largely on paste rheology and the subsequent paste extrusion settings. The viscosity of the paste when loaded into the extrusion syringe affects the formability, and more importantly shape sustainability of the filament that is extruded during the print procedure; a paste with too low viscosity (high solvent content) would result in a filament that is less likely to retain its shape, and more likely to deform upon settling on the built layer. On the other hand, solvent content in the prepared HA paste must be sufficient for formation of a strong weld between layers. If solvent content is too low, resulting in faster extrudate drying and insufficient weld formation, it can affect mechanical strength of the 3D printed scaffold.

Moreover, the SEF process can be used effectively to produce composite scaffolds. Synthetic bioactive and bioresorbable composite materials are becoming increasingly important as scaffolds for tissue engineering. Biomaterials should combine bioactive and bioresorbable properties to activate in vivo mechanisms of tissue regeneration, stimulating the body to heal itself and leading to replacement of the scaffold by the regenerating tissue. Bioceramics such as HA, TCP, and bioactive glasses react with physiologic fluids to form tenacious bonds with hard (and in

some cases soft) tissue. However, these bioceramics are relatively stiff, brittle, and difficult to form into complex shapes. In particular, HA possesses a biodegradation rate that is too slow, making it an unsuitable choice as a single material for bone scaffolds. Conversely, synthetic bioresorbable polymers are easily fabricated into complex structures, yet they are too weak to meet the demands of surgery and the *in vivo* physiologic environment. Composites of tailored physical, biologic, and mechanical properties as well as predictable degradation behavior can be produced combining bioresorbable polymers and bioactive inorganic phases [74].

p0110 PLA or PLGA/HA is considered as a composite biomaterial in which PLA/PLGA acts as the phase with a high biodegradation rate and HA provides strength to the composite. This can represent the effective optimal solution for tailored tissue engineering scaffolds, making tissue engineering a realistic clinical alternative in the near future. 3D printing of PLA, PLGA, and PLA/CaP have already been widely reported through several papers using melt extrusion freeforming [75,76], compression molding [77], and indirect AM [78] methods while there are very few reports on extrusion freeforming of PLA/CaP composites without material melting [79,80]. It has been found to be quite challenging to print PLA/CaP composite scaffolds with sufficient strut uniformity and resolution using low-temperature extrusion freeforming [80]. Highly uniform PLA/HA scaffolds could be printed by the authors using the SEF method and the preliminary results were promising. HA particles expose on the surfaces of the printed scaffold suitable for cell attachment (Fig. 10.2B). The addition of PLA allowed making flexible structures as elastic modulus of the printed filaments could be controlled by adjusting PLA percentage. The flexible printed PLA/HA layers could also be rolled out to make porous tubes (Fig. 10.2C) which are suitable structures with a significant number of areas that can be pursued.

p0115 Biopolymer/bioceramic composites such as PCL/TCP or PLA/HA are suitable for a significant number of applications. However, they still lack enough strength to be used for load bearing applications. There has been a trend in recent years to develop PEEK-based biocomposites due to PEEK's excellent cell biocompatibility and desirable mechanical properties such as strength, and elastic modulus; in addition to it being comparable to cortical bone [30,31]. Calcium phosphates including HA, β -TCP, or Bioglass, are utilized as a composite filler to produce PEEK compounds with potential for osseointegration [81,82]. In addition, incorporation of porosity into PEEK has been realized as an effective method to improve bone apposition [83,84]. Alternatively, surface modification has been used to enhance the mechanical and biological properties of PEEK [85]. Titanium and HA coated PEEK has also shown much higher bone-to-implant contact ratio than the pure PEEK implants [86,87].

p0120 Different processing methods such as compounding and injection molding, compression molding, cold press sintering and selective laser sintering (SLS) have been used to produce bioactive PEEK/HA and β -TCP composites [36–38]. SLS, a type of powder-based AM technology, is capable of fabricating bioactive PEEK/HA structures with very complex architecture permitting greater design freedom. The SLS technique has been hampered by difficulty in loading the quantity of bioactive

filler beyond 22% by volume, and exceeding porosity beyond 70–74 vol% [88]. The methods of bioactive PEEK processing discussed earlier do not permit control of distribution of the bioactive phase within the PEEK matrix. This limitation results from reliance of these techniques on mixing PEEK with bioactive material in powder or granular forms. In addition, the wide range of physical properties of these particles (i.e., size, shape, and density) typically hinders efficient and consistent mixing.

p0125 A novel technique was proposed by Vaezi and co-workers [89,90] that provides greater control on incorporation of bioactive materials into PEEK. The proposed technique integrates the SEF method and compression molding processes so that the bioactive phase is 3D printed and subsequently is overmolded with PEEK. The approach provides new possibilities to produce both bioactive PEEK compounds and porous PEEK structures with enhanced biological performance. Furthermore, this approach enables designers to control precisely the distribution of bioactive phase within the PEEK matrix and hence tailor biological and mechanical properties of the final composite. This is the first report of PEEK/HA composite displaying 100% interconnectivity of both the bioactive HA network phase and PEEK matrix which is superior to existing microstructural designs. The technique is versatile, such that a range of bioactive materials such as Bioglass, β -TCP, etc., with different rates of biodegradation can be used and the interconnected bioactive network can be fully absorbed in vivo, leaving 3D interconnected channels for further ingrowth and proliferation. Thus, a 3D locked bone/PEEK structure could be achieved in vivo, which can dramatically improve implant fixation compared with existing techniques.

p0130 **Fig. 10.3** illustrates SEM images of a typical PEEK/HA composites produced by Vaezi et al. [90]. As can be seen, HA scaffold with a pore size of 200 μm is fully infiltrated by PEEK in both vertical and lateral directions, while maintaining the HA network structure and uniformity.

f0020 **Figure 10.3** Typical bioactive PEEK/HA composite produced by overmolding of 3D printed HA scaffold with PEEK, HA filament size: 250 μm , pore size 200 μm ; (A) a vertical section, and (B) close view of PEEK–HA interface.

Source: Taken from M. Vaezi, C. Black, D.M.R. Gibbs, et al., Characterization of new PEEK/HA composites with 3D HA network fabricated by extrusion freeforming, *Molecules* 21 (2016) 687. doi:10.3390/molecules21060687.

Figure 10.4 Schematic of the use of 3D printed hierarchical HA scaffold with computer-controlled varied spacing suitable to make functionally graded PEEK/HA composites for spinal cage fusion.

A potential future application of these functionally graded PEEK/HA materials in spinal arthrodesis is shown in Fig. 10.4. As demonstrated, the greatest mechanical strength is achieved in the zone where HA has larger spacing (lower HA content), and enhanced biological performance in regions in contact with bone, where HA lattice has smaller spacing (greater HA content). Computer-controlled distribution of HA in these composites ensures uniform distribution of load onto the device after implantation as well.

In addition to bioactive PEEK compounds, the technique can be utilized to produce porous PEEK with fully interconnected pores and controlled porosity by soaking the PEEK/HA composite into hydrochloric acid (HCl) solution [90]. HA filaments are dissolves in HCl, resulting in hollow channels suitable for cell attachment, infiltration and proliferation. These channels have shown to be helpful for the alignment and differentiation of cells [91]. The use of the SEF method permits excellent control on pore size and interconnectivity which is necessary for bone-ingrowth.

10.5 High resolution 3D printing of bioceramics

Material extrusion technology is a very important part of extrusion-based AM systems. In 1993, John Benbow and John Bridgwater established a model of paste

Figure 10.5 Schematic diagram of paste extrusion based on Benbow–Bridgwater model.

flow and extrusion [92]. Schematic diagram of paste extrusion based on Benbow–Bridgwater model is as shown in Fig. 10.5.

Paste is contained in a barrel with diameter D_0 and is forced by a ram into a die land (capillary) with diameter D and length L . The important feature of flow in a capillary is the use of very small capillary diameters that flow and is laminar throughout with no turbulent core. So, the rheological properties from experimental measurements of a flowing paste can be determined. In proceeding from the barrel into the die land, the paste extends in the direction of flow and its cross-section decreases. There are two flow zones in the extrusion process using the Benbow–Bridgwater model: one is from the barrel into the die land and the extrusion pressure is expressed as P_1 ; the other is along the die land, with the extrusion pressure expressed as P_2 . Incorporating the two zones, the extrusion pressure required for paste flow can be calculated with Eq. (10.1), assuming that the paste material is thought to behave as a perfect plastic.

$$P = P_1 + P_2 = 2(\sigma_0 + \alpha V) \ln\left(\frac{D_0}{D}\right) + 4(\tau_0 + \beta V) \left(\frac{L}{D}\right) \quad (10.1)$$

However, it is often found experimentally that paste materials are not perfect plastics and exhibits some non-linear behavior, therefore, Eq. (10.1) can be modified as:

$$P = P_1 + P_2 = 2(\sigma_0 + \alpha V^m) \ln\left(\frac{D_0}{D}\right) + 4(\tau_0 + \beta V^n) \left(\frac{L}{D}\right) \quad (10.2)$$

In Eqs. (10.1) and (10.2), P is the extrusion pressure, σ_0 is initial bulk yield stress of the paste, τ_0 is initial paste-die wall shear stress, α is velocity-dependent parameters for the convergent flow on the die entry, β is velocity-dependent

parameters for parallel flow in die land, m and n are exponents, V is the mean extrusion velocity of the paste, and D_0 is barrel diameter, D and L are die land (capillary) diameter and length, respectively. Six characterizing parameters, namely σ_0 , α , m , τ_0 , β , and n , have three being associated with flow from the barrel into the die land and three with flow along the die land. The parameters σ_0 and τ_0 depend strongly on the paste formulation.

p0165 The unremitting drive toward printing materials at high resolution with faster speed gives rise to many opportunities and challenges. Lewis et al., leaders in extrusion-based 3D direct writing, reported the minimum feature size of $\sim 200 \mu\text{m}$ for ceramic inks [48]. The highest resolution ceramic lattice structure has been reported by Evans's and Yang's group [58] with filaments circa $70 \mu\text{m}$ printed using the SEF method. The key feature of the SEF method is its unique nozzle design, and paste formulation that allows very uniform pastes to be made quickly with excellent extrudability. The main challenges to decrease nozzle size with the aim of increasing ceramic 3D printing resolution are:

- u0010 • Increased extrusion force: scaling down the nozzle size for better resolution will make the required extrusion pressure unpractically high for high viscosity ceramic colloidal inks, since the extrusion pressure scales up much faster when the nozzle diameter is decreased according to the Hagen–Poiseuille equation [93].
- u0015 • Nozzle jamming or clogging due to dilatancy, agglomerates, impurity, process contamination, and quick drying of colloidal ink in outlet of nozzle.

p0180 According to the Eq. (10.2), die land length and ram velocity need to be selected as infrequently as possible while solvent content should be increased sufficiently (to decrease σ_0 , τ_0) to have minimum effect on extrusion pressure by decreasing die diameter with the aim of printing high resolution scaffolds. In addition, using a small syringe's barrel size can be useful for reduction of extrusion pressure (according to Eq. 10.2) and the paste needs to be prepared with a high level of stabilization (to avoid agglomerates) with enough lab work cares to avoid any contamination.

p0185 HA scaffolds with filaments as fine as $30 \mu\text{m}$ could be printed for the first time using a bespoke-developed SEF device by carefully tuning paste formulation for optimal paste consistency, extrusion speed (i.e., extrudate velocity), and using a unique nozzle design with minimum die land. Fig. 10.6 shows typical printed samples with filament diameter of 60 and $30 \mu\text{m}$. The sintering-induced micropores within a monofilament can be seen in Fig. 10.6B. Microporosity of HA scaffolds of various pore and filament sizes could be measured using Archimedes' method [90]. The microporosity calculated across samples lies within the range of 7%–10% with little variability, showing consistency in the properties of the material used to produce each sample and minimal presence of pores within the filaments.

p0190 Also, the uniformity of the printed scaffold with $60 \mu\text{m}$ (Fig. 10.6A) is considerably higher than the scaffolds with $30 \mu\text{m}$ filaments (Fig. 10.6D). This might be due to agglomerates within the HA paste which could result in a remarkable extrusion pressure fluctuation during print process. As local agglomerates can increase extrusion pressure, more paste is extruded in a short period of time after releasing the agglomerate which results in filaments with larger width. The dimensional

Figure 10.6 (A) HA scaffolds with 60 μm filaments printed using SEF method, solvent content: 10.3 wt%, die land 500 μm , extrusion pressure 13.2 MPa; (B) magnified image of fractured surface of HA monofilament with sintering-induced micropores; (C, D) a typical 4 layer 3D printed HA scaffold with 30 μm filament printed using SEF method, solvent content of 11.7 wt%, die land 30 μm , and average extrusion pressure of 17.9 MPa.

inaccuracy can also be due to inconsistency and variation in liquid content of paste due to liquid phase migration (LPM) phenomena using a low ram velocity. LPM can be described by faster movement of the liquid phase than the solid components in the paste flow process. Previous researchers reported there is normally a velocity threshold above which LPM doesn't occur [94]. A highly viscous binder system was used for printing the samples to minimize LPM and improve the stability of the paste. However, using a very low ram velocity (to decrease extrusion pressure) and more solvent content than a normal paste might result in LPM and thus, inconsistency of HA paste composition during extrusion. In addition, when using increased solvent content the printed filaments have less yield strength and are thus easier to deform after extrusion using relatively high XY table moving speed.

10.6 Conclusions

A tissue scaffold plays a very important role in the process of tissue engineering for the growth of new, or repairs of, defected tissue. A scaffold provides the necessary

support for cells to proliferate and maintain their differentiated functions, and its architecture defines the ultimate shape of a new organ. As such, without the presence of a scaffold the cells for growth would not have an appropriate medium in which to propagate and grow around, preventing the generation of new tissue. Extrusion-based AM systems are favorable to print scaffolds because of their ability to process a wide range of biomaterials, maintain great control on porosity, and pores interconnectivity which is important to allow the proper cell in-growth. Extrusion-based AM systems with and without material melting were reviewed comprehensively in this chapter. The application of the SEF technique in low-temperature production of scaffolds from various highly demanded ceramics such as alumina, zirconia, and HA for tissue engineering application was comprehensively investigated. In addition, the application of the SEF technique in production of biocomposite PLA/HA scaffolds and PEEK/HA structures was presented. In this chapter, a new technique based on extrusion freeforming and compression molding was presented for preparation of bioactive PEEK/HA composite. The technique provides a high level of control on the distribution of the bioactive phase.

s0040 **Acknowledgments**

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Further reading

- H. Shao, Y. He, J. Fu, et al., 3D printing magnesium-doped wollastonite/ β -TCP bioceramics scaffolds with high strength and adjustable degradation, *J. Eur. Ceram. Soc.* 36 (2016) 1495–1503.

NON-PRINT ITEM

Abstract

Different fabrication methods from traditional chemical engineering methods to advanced additive manufacturing (AM) are used for fabrication of tissue engineering (TE) scaffolds. The traditional techniques are subjected to limitations such as manual intervention, and inconsistent and inflexible processing procedures. In addition, the traditional techniques usually cannot control pore size, pore geometry, and spatial distribution of pores properly. To complement these limitations, there has been a trend in recent years to fabricate TE scaffolds using AM processes directly (3D printing of final scaffold) or indirectly (making negative scaffold to be used as a mold). In particular, extrusion-based AM systems have been widely used for fabrication of TE scaffolds due to their ability of processing different biomaterials, their possibility of manufacturing scaffolds in a cell-friendly environment, their high reproducibility and flexibility, and their simple process control in comparison with other AM techniques. In this chapter, the applications of extrusion-based 3D printing techniques are reviewed. In addition, an in-depth discussion on solvent-based extrusion freeforming (SEF) method, recent advances in printing bioceramic scaffolds, and its new application to make polyether-ether-ketone (PEEK)-based composites are presented.

Keywords: Additive manufacturing (AM); 3D printing; tissue engineering (TE); extrusion-based 3D printing