Freeform fabrication of nanobiomaterials using 3D printing

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DOI: 10.1533/9780857097217.16

Abstract: Nanobiomaterials play an important role in nanobiotechnology and have made a great contribution to biomedical research and healthcare. Recent progress in nanobiomaterials has increased demand for multidisciplinary approaches from physical, biological and engineering sciences. Solid freeform fabrication (SFF) technologies are based on layer-by-layer deposition of materials which bring about new application possibilities for processing nanobiomaterials. The aim of this chapter is to provide a comprehensive overview of SFF techniques suitable for processing nanobiomaterials; current state and limitations regarding the techniques are discussed. Overviews of subjects including biofabrication of tissue engineering (TE) scaffolds using extrusion-based freeforming and dry powder printing of nanobiomaterials are also provided.

Key words: solid freeform fabrication, nanobiomaterials, extrusion freeforming, 3D printing, dry powder printing.

2.1 Introduction

Recent advances on nanotechnology have created new frontiers, terminologies, and possibilities which have led to breakthroughs in several distinct and multidisciplinary sciences. In particular, recent developments in the field of nanobiotechnology have significantly improved the area of nanomedicine, biomedical and healthcare sciences. Conventional biomedical applications have taken advantage of nanotechnology science in different areas such as intelligent systems, controlled release systems, tissue engineering (TE), biosensors and nanocomposites used in orthopaedic implants (Bartolo and Bidanda, 2008). Unique and useful characteristics over conventional materials have been observed from nanobiomaterials due to the size and surface effects that can be employed in various medical applications. Improved reactivity, bioactivity, electrical and optical properties, strength and magnetic characteristics are of particular interest to the biomedical field (Ferrari, 2005; Qin et al., 1999; Vasir et al., 2005). Moreover, there is the possibility of controlling some other properties
such as melting point and solubility of nanobiomaterials by altering their particle size.

Nanofillers such as nanofibres and nanoparticles (NPs) have been widely presented in different biomedical fields with new applications in mind. Polymeric nanofibres with unique and intrinsic properties, resulting from their high surface area to volume ratio, are attractive for many practical applications, and intensive studies have been conducted on this class of nanobiomaterials (Hasirci et al., 2006; Huang et al., 2003; Zhang et al., 2005). It has been observed that human cells tend to attach, grow and proliferate on fibres with diameters smaller than those of cells (Teixeira et al., 2003). In this way, polymeric nanofibres have great potential to be exploited in TE scaffolds, and their intrinsic properties and dimensions make them attractive carriers. The use of polymeric nanofibres in cartilage, nerve, bone, skin, skeletal muscle and blood vessel TE have being extensively studied (Mengyan et al., 2005; Xu et al., 2004; Yoshimoto et al., 2003; Zong et al., 2005). Nanofibres and NPs can also be exploited to reinforce the composite structures of various biomedical applications such as dental restorations (Chen et al., 2006) and production of orthodontic composites with enhanced elastic modulus and flexural and tensile strengths (Fong, 2004; Price et al., 2003). Reinforcement of composites using either nanofibres or carbon nanotubes (CNTs) has been of great interest in recent years. Exploitation of different NPs in hard tissue implants for the purpose of reinforcement is an interesting subject that is under development. Carbon nanofibres (CNFs) have been employed to reinforce poly (ether ether ketone) (PEEK) structures, and nanocomposites with superior properties were obtained (Sandler et al., 2002).

Advances in nanobiomaterials and the advent of new possibilities and applications have led to demands for the development of new fabrication and processing techniques, as conventional techniques are inefficient or unable to meet new requirements. Broad ranges of processing technologies have been developed with different applications and capabilities as their fundamentals are very diverse. Among different attainable methods, SFF processes based on layer-by-layer manufacturing are identified as effective approaches worth further investigation. SFF is a fabrication technique used for building three dimensional (3D) parts layer-by-layer directly from computer-aided design (CAD) data in a short time. The combined potential of nanobiomaterials and SFF technologies has been an exciting route in nanobiotechnology and health sciences over the past decade.

More than 30 different SFF methods are being exploited worldwide in various industries; around 20 are able to process biomaterials and have found biomedical applications (Chua et al., 2004). In addition to the processing of biomaterials, there has been another significant trend in processing a variety of nanobiomaterials using SFF methods. The additive nature of SFF
technologies ensures minimal waste of scarce and expensive nanobiomaterials. The use of CAD data enables fabrication of customized parts from nanobiomaterials, offers a high level of control over the architecture, and guarantees reproducibility. In addition to the complex shape of parts, the composition of the nanobiomaterials can be controlled in the parts, resulting in the potential to produce a variety of bio-nanocomposites efficiently. However, a specific class of SFF methods is currently suited for direct processing of nanobiomaterials (Fig. 2.1). Processes such as stereolithography (SL), nanocomposite deposition system (NCDS), selective laser sintering (SLS), inkjet printing, aerosol jet printing and extrusion-based systems are among the most widely used SFF approaches for nanobiomaterials processing. Moreover, SFF techniques can be used indirectly (building negative parts to be used as a mould) to produce final parts from nanobiomaterials (Dong-Woo et al., 2007).

In this chapter the key SFF methods suited for the direct processing of nanobiomaterials are discussed comprehensively, and emphasis will be placed on recent advancements in the respective techniques as a working principle of each process described in Chapter 1. In addition, the use of extrusion-based freeforming in TE scaffold fabrication, and nanobiomaterials dry powder printing are reviewed extensively.

### 2.2 Laser-based solid freeform fabrication (SFF) techniques

The key SFF systems suitable for direct processing of nano-biomaterials can be classified into three main groups (Fig. 2.1). The first group is laser-based SFF techniques including SL and SLS processes. This section provides a comprehensive overview on these processes and their current applications and possibilities.

![SFF techniques for nanobiomaterials processing](image)

- **Laser-based systems**
  - Stereolithography (SL)
  - Selective laser sintering (SLS)

- **Droplet-based systems**
  - Inkjet printing process
  - Aerosol jet printing

- **Nozzle-based systems**
  - Extrusion-based SFF techniques (see Section 2.6)
  - Nanocomposite deposition system (NCDS)

2.7 Classification of different SFF systems suitable for processing of nanobiomaterials.
2.2.1 Stereolithography (SL) process

Stereolithography was the first commercial SFF process developed by 3D Systems Inc. and is based on layer-by-layer polymerization of photosensitive resin using ultraviolet (UV) light. Two main SL techniques, namely scanning SL and projection SL, have been developed depending on the beam delivery system. Scanning SL solidifies the photopolymer (including UV photo-initiator, monomer and other additives) in a point-by-point and line-by-line style in each layer. In projection SL, build time is saved significantly as whole layer of the photopolymer is cured at once via exposure through the provided mask. A digital micro-mirror device (DMD), embedded in digital light processing (DLP) projectors, is normally applied as the dynamic mask in projection SL systems.

The SL process can be used for fabrication of 3D nanocomposite parts from resins, based on the insertion of a high load of bio-nanofilizers in a photosensitive polymer matrix which acts as the binder material. Use of resins containing nanofilizers in the SL process is mostly for the purpose of reinforcing the final nanocomposite part. The use of NPs with lower density and smaller particle size is associated with an increase in resin viscosity (Bartolo and Gaspar, 2008; Gaspar et al., 2008). The resulting nanocomposite objects produced in this way can also be subjected to debinding (via an appropriate thermal treatment) and sintering steps to be converted into pure 3D part. During the debinding and sintering steps, the shape of the part remains unaltered but the part is subjected to shrinkage. The load of nanoparticle in the resin should be controlled accurately and should be sufficiently high (up to 80 wt.% for alumina NPs) to avoid part deformation and crack generation. Nanoparticle content should be high enough (solid loading more than 50 wt.%) to obtain satisfying characteristics in the final, dense part. However, increasing nanoparticle content results in increasing viscosity of the suspension, and subsequently it becomes difficult to recoat the suspension layers during the SL process. Therefore, innovative approaches either using special recoating or through developing a low viscosity suspension are required to build parts from high NPs-loaded resin in the SL process. Instead of trying to develop low viscosity suspensions, Doreau and co-workers (2000) used a special scraper, patented by Optoform Inc., to spread a paste containing a photo-curable resin, high loads of ceramic particles (50–60 vol.%), dispersants and a thickener. In a similar way, Bertsch et al. (2004) gained success in processing a special paste containing very high loads of alumina NPs (up to 80 wt.%), a UV photoinitiator, a low viscosity monomer (Polyethylene glycol 400 diacrylate) and a silane (3-glycidoxypropyltrimethoxysilane) in the SL process. Silane served to prevent alumina particle agglomeration and to stabilize the formulation. As mentioned before, parts produced in this way keep their shape and show no
deformations or cracks once sintered if high ceramic NP loading is used, but some shrinkage and a residual porosity have been observed. Figure 2.2 depicts two different micro parts built by Bertsch and co-workers from alumina NPs with 50 wt.% and 75 wt.% loading.

Different research groups have fabricated parts by inserting nano-/micron-sized ceramic particles such as silica and silicon nitride (Griffith and Halloran, 1996), hydroxyapatite (HA) (Griffith et al., 1995), alumina (Greco et al., 2001; Hinczewski et al., 1998), etc., into water-based or acrylate-based, photocurable resins. Acrylate prepolymer such as 1,6-hexanediol diacrylate (HDDA, a low viscosity acrylate monomer) is normally used as matrix for alumina and HA NPs. An appropriate dispersant needs to be included to prevent nanoparticle agglomeration and to decrease the viscosity of the prepolymer.

In addition to NPs, CNTs can be exploited as nanosized fillers with the aim of improving mechanical properties of nanocomposites made by SL. Sandoval et al. (2007) dispersed controlled amounts of multiwalled carbon nanotubes (MWCNTs) in epoxy-based resins and made complex 3D nanocomposite parts with enhanced mechanical properties. Their electron microscopy results showed affinity between the constituents of the nanocomposite. In the meantime, buckled and collapsed MWCNTs in several micrographs of samples that were previously pulled in tensile tests were observed (Fig. 2.3). It was thought that the buckling and collapsing phenomena of the MWCNTs were a result of the photopolymerization (in the SL machine and in the UV oven) and thermal effects introduced by the SL system laser.
Stereolithography has been used directly and indirectly to make biodegradable TE nanocomposite scaffolds. Jin Woo et al. (2009) used a suspension containing poly (propylene fumarate) (PPF), diethyl fumarate (DEF) (to reduce the viscosity) and HA NPs to fabricate a nano-/micro-scale PPF/DEF-HA composite scaffold directly (Fig. 2.4c and 2.4d). In addition, they produced a negative scaffold model (Fig. 2.4a) as a mould with an internal pore size of 250 μm and a line width of about 350 μm from SL5180 resin (Huntsman). Then the mould was filled with HA nanopowders of 500 nm particle size, and the final scaffold was produced through a sintering process (Fig. 2.4b) (Dong-Woo et al., 2007).

2.2.2 Selective laser sintering (SLS) process

Selective laser sintering utilizes a CO₂ laser beam to sinter thin layers of polymers or their composite powders selectively to build 3D parts. Different biomaterials ranging from biopolymers and bioceramics to various biocomposites have been processed by SLS for possible medical applications. Powders outside the part boundary fuse during processing because of ‘growth’ effect phenomena that result in inaccuracy and rough parts with micropores on the surface which may promote cell attachment and growth (Yang et al., 2002). However, the powdery surface of SLS parts induces some difficulties in terms of sterilization and cell culture.

SLS has been recognized as a useful tool initially for fabrication of bone implants from poly (methyl methacrylate) coated calcium phosphate (Lee and Barlow, 1993), and further it was used to process some new high
performance biomaterials such as HA-reinforced polyethylene composites for bone implants (Hao et al., 2006). In particular, SLS, along with fused deposition modelling (FDM), have been recognized to be advantageous for fabrication of TE scaffolds among various SFF technologies because of their ability to process different kinds of biocompatible and biodegradable materials. Non-biodegradable polymers including ultrahigh molecular weight polyethylene (UHMWPE) (Rimell and Marquis, 2000) and PEEK (Schmidt et al., 2007) have been employed to build TE scaffolds. As for biodegradable polymers, scaffolds from poly (e-caprolactone) (PCL) (Williams et al., 2005) and poly (L-lactic acid) (PLLA) (Tan et al., 2005) have been produced using SLS. Moreover, different composite scaffolds (biodegradable polymers and bioactive ceramics) including HA/PCL, HA/poly (L-lactide-co-glycolide) (PLGA) and β-tricalcium phosphate (β-TCP)/PLGA have been produced using SLS (Simpson et al., 2008; Wiria et al., 2007).

Bio-nanocomposites comprising biopolymer and different types of nanofillers are of particular interest with the SLS process as significant changes
in biological or mechanical properties can be obtained with the use of only a small amount of nanofillers. Nanofillers in the form of NPs or nanofibres are used to control biodegradability and bioactivity due to the high surface to volume ratio. By using nanofillers some other properties such as mechanical properties, optical properties, thermal conductivity and heat resistance can be enhanced. NPs such as nanosilica (Chung and Das, 2008) and nanoalumina (Haizhong et al., 2006) are commonly used within a biopolymer matrix to improve the mechanical properties of nanocomposites. Nanofillers can offer improved strength in the x–y direction of a part, but typically offer little or no additional strength in the z-direction as they do not span the divide between build layers. Positive effects such as decreasing the required laser energy have been observed using nanofillers specifically due to the fact that they can absorb laser power more efficiently (Tolochko et al., 2000). Ho and co-workers (2002) showed that graphite fillers have the most significant effect on improving the absorptance of the laser sintering polycarbonate (PC) powder among other examined fillers, including quartz, silica and talc, since it was proposed that graphite powder could minimize thermally related problems since less laser energy is required for sintering and less energy is transmitted through the graphite powders.

Uniform base powder (as matrix), nanofiller distribution and good interfacial adhesion between them are two very important factors in SLS achieving a high performance nanocomposite part (Jain et al., 2010). Mechanical mixing of the filler with the base powder is normally used for most biopolymer nanocomposites. However, mechanical mixing does not seem to be a sufficiently effective approach for uniform mixing of two powders with different sizes (especially when one is nanosized) and different densities (e.g., biopolymer and metallic nanofiller). Nanofillers can be coated with the base polymer for homogeneous dispersion and preventing nanofiller accumulation.

Calcium phosphate (Ca-P) nanofillers including HA and TCP NPs and nanofibres have been considered widely in the development of biomaterials in recent years due to their osteoconductivity, nano size effects and biomimetic resemblance to natural bone structure when mixed with biopolymers such as chitosan, collagen and PLLA. Biodegradable, osteoconductive nanocomposite scaffolds for bone tissue regeneration comprising a biodegradable polymer matrix such as PLLA and poly (hydroxybutyrate–co-hydroxyvalerate) (PHBV) with bioactive Ca-P nanofillers have been successfully built via a SLS process (Bin et al., 2010). Cheung et al. (2008) used carbonated hydroxyapatite (CHAp) NPs within a PLLA matrix to produce nanocomposite TE scaffolds using a modified SLS machine (Fig. 2.5).

In an interesting study, Lin and co-workers (2009) used CNTs as filler and β-TCP NPs (average particle size 20.1 nm) as the main material to produce bone TE scaffolds with enhanced mechanical performance. β-TCP NPs,
binder materials (particle size ~110 μm) and CNTs with the quality percentage of 0.1%, 0.2% and 0.3% individually were mixed using a four tank mixer. By increasing the amount of CNTs gradually, the scaffold strength first increased and then decreased. Results showed that the strength of scaffold mixed with 0.2% CNTs reached 0.819 MPa, which is an improvement of 85.7% compared with that without CNTs. The reduction in composite materials strength is thought to be caused by non-uniform dispersion of CNT aggregates. CNTs have a large aspect ratio, high surface energy and can easily form aggregates in the matrix material. Ko et al. (2007) proposed that the combination of SLS and inkjet printing processes would be an asset

![Image of PLLA/CHAp nanocomposite scaffold produced by SLS.](image_url)

![Image of the layer structures of a PLLA scaffold.](image_url)

![Image of the layer structures of a PLLA/CHAp nanocomposite scaffold.](image_url)

2.5 (a) PLLA/CHAp nanocomposite scaffold produced by SLS. (b) SEM image of the layer structures of a PLLA scaffold. (c) SEM image of the layer structures of a PLLA/CHAp nanocomposite scaffold. It can also be seen from Fig. 2.4b and 2.4c that the degree of fusion of the PLLA/CHAp nanocomposite is lower than that of the pure PLLA powder as the CHAp nanoparticles on the powder surface might act as a barrier against fusion (WenYou et al., 2008).
to increase resolution of the existing bio-nanoparticle inkjet printing. They set up a device for SLS of inkjet-printed Au nanoparticle solution on a polymer substrate by scanning with a focused continuous laser.

2.3 Droplet-based SFF techniques

The second group of SFF systems suitable for processing of nano-biomaterials is droplet-based SFF techniques including inkjet printing and aerosol jet processes. In this section, the principles and the recent progress of each process toward the processing of nano-biomaterials are described.

2.3.1 Inkjet printing process

In recent years there has been a propensity to mutate inkjet printing into a tool that can be applied in different manufacturing processes such as soldering microelectronics or fabricating micro-optical components using photocurable resins. Furthermore, inkjet printing technology has been used in a layer-by-layer process for direct freeforming of complex 3D structures pioneered by Evans and his group. In inkjet printing, liquid material (in droplet form) often turns into solid following the deposition process via cooling (e.g., by crystallization or vitrification), chemical changes (e.g., through the cross-linking of a polymer) or solvent evaporation (Hon et al., 2008). Two different modes are prevalently utilized for droplet creation, including drop on demand (DOD) and continuous inkjet (CIJ). Generally, CIJ systems use fluids with lower viscosity at higher drop velocity than DOD and are mostly used where printing speed is important. In contrast, DOD is used where smaller drop size and higher accuracy are required, and it has fewer limitations on ink properties as compared with CIJ. In DOD, ink droplets are ejected from a reservoir through a nozzle using an acoustic pulse which can be induced either thermally or piezoelectrically. In thermal DOD, a vapour bubble which is generated by local heating of the ink causes droplet ejection. Thermal DOD is greatly restricted to using water as a solvent and thus compels strict limitations on the number of polymers that can be processed (de Gans and Schubert, 2003). In piezoelectric DOD, deformation of a piezoelectric membrane results in generation of acoustic pulses, and consequently ejection of the droplets. Piezoelectric DOD is an appropriate technique for a variety of solvents, and thus suited for different nanobiotechnology applications.

Inkjet printing of ceramics using both piezoelectric and thermal printers has been reported for various 3D micropatterning applications such as creating internal cavities (Mott et al., 1999), functional gradients (Mott and Evans, 1999) and arrays of pillars (Evans et al., 2001, Lejeune et al., 2009).
Piezoelectric DOD printers that print molten waxes at about 120°C have also been used to deposit suspensions with up to 40 vol.% ceramic powder (Seerden et al., 2001). Figure 2.6 depicts some micropatterns produced by ceramic inkjet printing.

The physical properties of the chosen ink are probably the most vital aspects of inkjet printing. Viscosity, surface tension and inertia are the three main factors which affect behaviour of droplets and liquid jets. The viscosity of ink should be adequately low since the power produced by the piezoelectric diaphragm is limited. On the other hand, surface tension should be sufficiently high to avoid ink dripping from the nozzle. Some dimensionless parameters such as Reynolds number \((Re)\), Weber number \((We)\) and Ohnesorge number \((Oh)\) are used for describing and analysing jetting and breakup phenomena in droplet generation. The Reynolds number is a characteristic which describes the ratio between inertial and viscous forces and is obtained by \(Re = \rho dv/\eta\), where \(\rho\) is fluid density, \(d\) is specific length (droplet diameter), \(v\) is fluid velocity and \(\eta\) is dynamic viscosity. Weber number
is a characteristic which describes the ratio between kinetic energy and surface energy and is obtained by $We = \frac{\rho dv^2}{\sigma}$, where $\sigma$ is surface tension. In addition, the Ohnesorge number is a characteristic which describes the relative importance of viscous and surface forces and is obtained by $Oh = \frac{We^{1/2}}{Re} = \frac{\eta}{\rho \sigma d}^{1/2}$ (Hon et al., 2008). According to research work by Wang and Derby (Tianming and Derby, 2005), for $Oh > 1$ fluid viscous dissipation results in nozzle clogging and impedes ejection of drops and also for $Oh < 0.1$ multiple drops are produced instead of a single, well-defined drop. So in practice, jettability criterion for precision DOD printing is $1 > Oh > 0.1$ and correspondingly droplet velocity should be 5–10 m/s. It should be noted that for non-Newtonian fluids other parameters such as the Weissenberg number ($Wi$) are used to consider the effects of viscoelasticity. The $Wi$ value can be obtained from $Wi = tv/d$, where $t$ is a characteristic relaxation time of droplet (Hon et al., 2008).

There has been a significant trend towards inkjet printing of inks containing bio-NPs in recent years. A large proportion of the atoms is in the surface of NPs which results in favourable properties such as a reduction in the melting point of metal biomaterials. The size of NPs should normally be 100 times less than the diameter of the jetting nozzle to avoid nozzle clogging (Kosmala et al., 2011). At the same time, ink containing bio-NPs should be non-viscous, and volatility of solvent should be adequately low to prevent nozzle clogging. Figure 2.7 shows a typical experimental set-up for printing inks containing NPs using piezoelectric DOD. The build plate can move in x and y directions and NP droplets can be observed via a CCD camera. In such a DOD experimental system, droplets are ejected via voltage waveform changes (Fig. 2.7, inset diagram). In short, the first rising voltage expands the glass capillary and a droplet is pushed through the nozzle due to the falling voltage. The final rising voltage cancels some of the residual acoustic oscillations that remain after droplet ejection and may cause satellite droplets.
The CCD camera captures images at the droplet generation frequency (Ko et al., 2010).

Droplet formation, its break-up and corresponding tail are related to the viscosity of the nanoparticle-based ink; the shape of the droplets (i.e., spherical) is influenced by surface tension. As for inks containing bio-NPs, viscosities in the range of 2–30 mPa s and surface tensions up to 60 mN/m are acceptable (Magdassi, 2010). In the meantime, proper substrate temperature allowing sufficient drying of the bio-nanoinks in each layer is essential for successful 3D printing. In short, the basic conditions required for successful 3D bio-nano inkjet printing are: ink properties (viscosity, surface tension); jetting parameters (signal width, voltage magnitude, jetting frequency); and environment (pressure, environmental and substrate temperature, humidity) (Ko et al., 2010).

Overcoming the strong agglomeration of the NPs in solution is the main challenge in making printable and stable bio-nanoink. Basically, nanoparticles tend to aggregate and cluster which results in fewer, larger particles in the solution, viscosity increment and fluctuation during storage. Viscosity measurement during storage time is normally performed to determine agglomeration rate and to investigate stability of nanoinks. A well-dispersed nanoink should be stable for at least one week at room temperature with no particle sedimentation. Some surface modifications on bio-NPs can be performed to avoid or delay aggregation of particles. For example, gold nanoparticles have been protected via coating with two different polymers, namely, poly(vinylpyrrolidone) (PVP) and acrylic resin on the surface of the particles to make the ink stable for a long time (1 year) even at gold concentration higher than 20% (Wenjuan et al., 2010).

To date, different bio-nanoinks have been successfully processed by inkjet printing processes. Nanobioceramic ink containing nanotitanium dioxide (TiO$_2$) has been inkjet printed on glass substrate (Hosseini and Soleimani-Gorgani). Gold (Fuller et al., 2002) and silver (Kosmala et al., 2011) NPs have been extensively investigated for inkjet printing. Hwan Ko et al. (2010) used ink containing gold NPs to produce true 3D parts including micro-pillar arrays, micro-helix, and micro-zigzag using linear and rotary tables. Inkjet printing of nanoinks containing single-wall carbon nanotubes (SWCNTs) (Chen et al., 2010; Nobusa et al., 2011, Song et al., 2008), MWCNTs (Kordas et al., 2006) and graphene (O’Connell et al., 2008) has also been reported. SWCNT and graphene are normally dispersed in dimethylfolmamide (DMF) (Song et al., 2008) and dichloroethane (DCE)/poly (mphenylenevinylenecoco-2,5-dioctoxy-p-phenylene) (PmPV) (O’Connell et al., 2008) suspensions, respectively, to make a stable ink for inkjet printing. In the meantime, sonication and centrifugation should be applied to remove heavy particles. Inkjet printing of antibiotic- and calcium-eluting 2D micropatterns was explored by Yexin et al. (2012) as a novel approach for
facilitating osteogenic cell development on orthopaedic titanium implant surfaces and preventing the formation of biofilm colonies. Using a commercial inkjet printer (Dimatix Materials Printer, DMP2800, FujiFilm Dimatix, Santa Clara, CA), circular dots with ~50 μm diameter were printed in arrays with ~150 μm distance from inks containing rifampicin (RFP) and PLGA dissolved in an organic solvent with ~100 nm biphasic calcium phosphate (BCP) NPs suspended in the solution.

2.3.2 Aerosol jet process

The aerosol jet process is a type of direct writing method which uses a focused aerosol stream instead of liquid ink droplets (as is used in inkjet printing) to deposit a wide range of materials. The process was developed and commercialized by OPTOMEC® under the trademark of M3D which stands for Maskless Mesoscale Material Deposition. Figure 2.8 depicts a schematic of the aerosol jet printing process. First, composite suspension is aerosolized in an atomizer to make a dense aerosol of tiny droplets (normally 1–5 μm in diameter but droplets as fine as 20 nm have been obtained). Next, the aerosol is transported to the deposition head via a carrier gas flow (usually N₂ gas flow), and within the aerosol head, the aerosol is focused using a flow guidance deposition head, which creates an annular flow of sheath gas to collimate the aerosol. The high velocity co-axial aerosol stream is sprayed onto a substrate layer by layer (minimum layer thickness of 100 nm) to create 3D parts (Hon et al., 2008). The high exit velocity of the aerosol stream enables a relatively large separation between the print head and the substrate, typically 2–5 mm. The aerosol stream remains tightly focused over this distance, resulting in the ability to print conformal patterns on 3D substrates. Writing speeds of up to 200 mm/s, line widths from 5 μm to 5 mm, inks with viscosity from 0.7 to 2500 mPa s and maximum

2.8 Schematic illustration of aerosol jet process. (Source: Courtesy of OPTOMEC Inc.)
volumetric deposition rate of 0.25 mm³/s have been reported. Depending on the ink and substrate materials used, furnace, infrared laser and UV-curing (for polymers) can be used post-processing to achieve the desired mechanical and electrical properties.

Since aerosol jet printing is a low temperature process and the droplet size is of the order of a few femtolitres, it is a good candidate for biomanufacturing. The kinetic energy of droplets is so small that it will not demolish living cells due to their tiny mass. Aerosol jet inks can include polymers, ceramics, metals and biomaterials in the form of solutions, nanoparticle suspensions, etc. Materials including metals (bio-nanoinks containing Ag, Au and Pt NPs, Pd and Cu inks), resistors (carbon polymer thick film (PTF), ruthenium oxide), dielectrics (polyimide, polyester, polytetrafluoroethylene (PTFE), etc.) and biomaterials (such as protein and antibody solutions, DNA and biocompatible polymers like PLGA) have been employed successfully in the aerosol jet process (Hon et al., 2008).

Aerosol jet printing was first developed for 2 and 2.5D direct writing purposes, but with recent process developments there is possibility of using this process efficiently for true 3D nanobiomaterials manufacturing. Typical characteristics of nanoparticle based inks for aerosol jet systems are: solvent with low evaporation rate; NP size should be less than 500 nm (<200 nm preferred); solids content within the range 5–70 wt.% is possible; and viscosity of ink should be within the range of 1–1000 cP at ambient temperature. Aerosol jetting has been used successfully to produce bioceramic/polymer nanocomposite scaffolds for bone TE applications. Liu and Webster (2011) reported the use of an aerosol jet process for fabrication of 3D nanostructured titania/PLGA nanocomposite scaffolds for orthopaedic applications. In vitro cytocompatibility tests were conducted and the results demonstrated that the 3D nanocomposite scaffold they produced enhanced osteoblast infiltration into porous 3D structures in comparison to previous nanostructured surfaces. SWCNT inks have been formulated successfully as well for different biomedical applications.

### 2.4 Nozzle-based SFF techniques

The third group of SFF systems suitable for processing of nano-biomaterials is nozzle-based SFF techniques. This section will address these processes and provide detailed descriptions of their applications and their main advantages and limitations

#### 2.4.1 Nanocomposite deposition system (NCDS)

The NCDS process was developed by Won-Shik and co-workers (2007) to overcome the recoating problem of nanofiller-loaded resin in SL. This process
uses a nozzle to deposit and cure bio-nanoparticle filled biodegradable resin layer by layer. NCDS is a hybrid SFF technique which consists of two main operations in each layer, including nanoparticle-filled resin deposition and further material removal using micro-machining. Biocompatible UV-curable resins are used as matrix and various bio-nanofillers are used to form composite materials. Figure 2.9 shows a typical NCDS hardware and schematic NCDS process sequence.

The machine (1) deposits a thin layer of UV-curable resin containing bio-nanofillers onto the substrate using a micro-needle, (2) solidifies the deposited resin via UV ($\lambda = 365$ nm) and (3) removes the unnecessary deposited materials via micro-machining. The nanocomposite can be deposited into 10–100 μm layers to produce a near-net shape and further precise micro-machining is used to obtain the net shape. NCDS shows anisotropic compressive properties like some other SFF processes such as FDM and 3D printing (Ahn et al., 2007b).

Different nanobiomaterials have been processed by NCDS to produce nanocomposites. Biocompatible acrylated polyurethane resin is normally used as a matrix of nanocomposite with different nanofillers such as HA.
NPs and MWCNT, etc. Ahn and co-workers (2007) mixed acrylated polyurethane resin with HA NPs with an average diameter of ~100–300 nm and MWCNT with an average diameter of about 40 nm and an aspect ratio of over 1000. Both composite mixtures had a viscosity near 100 000 cPs (±20 000), which could flow through the micro-needle when pressurized by the dispensing system. Nanocomposite scaffold-type drug delivery devices have also been fabricated with the use of PLGA as a biodegradable base polymer, HA NPs as a release modifier and 5-fluorouracil (5-FU) as a model drug (Chu et al., 2007; Lee et al., 2008; Park et al., 2010).
2.4.2 Extrusion-based SFF systems

The term ‘direct ink writing’ was first proposed by Lewis (Lewis and Gratson, 2004) for a class of manufacturing techniques that utilize a computer-controlled ink deposition nozzle to create patterns and 3D objects with controlled composition and architecture. Direct ink writing techniques are classified into two main sub-groups: extrusion-based and droplet-based systems. Droplet-based systems deposit inks in the form of droplets to form final patterns and shapes. Inkjet printing and aerosol jet writing discussed earlier are the two main droplet-based direct ink writing systems. Extrusion-based systems have the same working principle barring that they deposit material in a continuous flow and are characterized by an extensive diversification.

Extrusion-based systems can basically be classified into two main sub-groups as shown in Fig. 2.10: processes based on material melting and processes without material melting. Fused deposition modelling, precision extrusion deposition (PED) (Wang et al., 2004), 3D fibre deposition (Woodfield et al., 2004), precise extrusion manufacturing (PEM) (Xiong et al., 2001) and multiphase jet solidification (MJS) (Greulich et al., 1995) are SFF techniques based on material melting. Pressure-assisted microsyringe (PAM) (Vozzi et al., 2002); low-temperature deposition manufacturing (LDM) (Zhuo et al., 2002); 3D-bioplotting (Landers and Mulhaupt, 2000); robocasting (Cesarano, 1999); direct-write assembly (pH-controlled gelled ceramic colloid or polymers freeforming) (Smay et al., 2002); and solvent-based extrusion freeforming (Grida and Evans, 2003) are the most commonly used SFF 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 the two main nozzle designs including filament driving wheels, and mini-screw extruder have been used in processes with material melting. More details on different extrusion-based free-forming techniques are provided in Section 2.5.2.

Much attention has been paid to extrusion-based systems in recent years as they are mechanically simple processes in comparison to other SFF techniques, and a wide range of biomaterials can be processed effectively. Different nanofillers have been employed to improve mechanical properties, bioactivity, etc. Nanofibre-reinforced biopolymers are fabricated by FDM so that nanofibres are well distributed in the matrix with no porosity in the bio-nanocomposite. Pressure-assisted microsyringing has been used to fabricate nanoHA/PCL bio-nanocomposite bone TE scaffolds (Heo et al., 2009). The use of HA NPs (particle size 20–90 nm) resulted in a higher compressive module and better attachment and proliferation of MG-63 cells in comparison to composite PCL scaffold fabricated using micron size HA particles and conventional PCL scaffold. Ye et al. (2010) reported the use of a PED technique to build bone bio-nanocomposite scaffold from nano-non-stoichiometric apatite (ns-AP) and PCL matrix. Jinku et al. (2012) used FDM to build PLGA/\(\beta\)-TCP and HA nanocomposite scaffolds. Mattioli-Belmonte et al. (2012) fabricated PCL/CNT composite scaffolds using a PAM process and reported the mechanical, thermal and biological characterization of the produced scaffold in which both the quantity of nanotubes in the matrix as well as the scaffold design were varied in order to tune the mechanical properties of the material. Their PCL/CNT nanocomposites were able to sustain osteoblast proliferation and modulate cell morphology. Their work shows the potential of custom designed CNT nanocomposites for bone TE. Dorj et al. (2012) used robocasting to produce a novel nanocomposite scaffold made of chitosan and nanobioactive glass (nBG) retaining a dual-pore structure. Robocasting was carried out under a cooled bath containing dry ice, to rapidly solidify the scaffold because the dispensed solution was hard to solidify at ambient conditions. The chitosan/nBG nanocomposite scaffolds were well-constructed, with an aligned, macro-channelled pore structure (Fig. 2.11a). As seen in Fig. 2.11b, the scaffold filament presented a highly microporous structure, with pore sizes of a few to 10 \(\mu\)m, demonstrating micro/macropore dual-pore structure. Closer examination of the surface revealed the presence of the nanofibrous component of the nBG (arrowed in Fig. 2.11c) embedded within and enclosed by the chitosan dense matrix. The pure chitosan scaffold robocast under the same conditions as the chitosan/nBG scaffold also showed a highly microporous and macro-channelled structure (Fig. 2.11d). The chitosan micropores were shown to be more elongated and a little larger than those of the nanocomposite scaffold (Fig. 2.11e) (Dorj et al., 2012).
2.5 Extrusion freeforming of biomaterials scaffold

One of the principle methods behind tissue engineering involves growing the relevant cells in vitro into the required 3D organ or tissue. However, cells are unable to grow in favoured 3D orientations and thus define the anatomical shape of the tissue. Instead, they randomly migrate to form a two-dimensional (2D) layer of cells. Scaffold is a porous structure that acts as a synthetic extracellular matrix (ECM), which permits cells to grow in favoured orientations and facilitates cell adhesion, proliferation and differentiation (Sachlos and Czernuszka, 2003). In the following sections the main procedures for scaffold design and fabrication using extrusion freeforming techniques will be described.

2.5.1 Scaffold materials and macro-microstructure design

One of the most important challenges in TE is to design an ideal scaffold. In addition to mimicking the structure and biological functions of ECM, TE scaffolds should provide a good environment for cell attachment,
proliferation and differentiation. In this way, several aspects should be taken into consideration in the design of scaffolds for TE. In addition to being biocompatible (both in bulk and degraded form), TE scaffolds should provide a correct stress environment for the neotissues through their appropriate mechanical properties. Scaffolds also need to be sufficiently porous to allow the introduction of cells and nutrients and removal of waste materials, and should possess an appropriate surface chemistry for cell attachment. Two main aspects for scaffold design are scaffold material and scaffold macrostructure.

Selection of suitable biomaterials for fabrication of the TE scaffolds is a significant step as the scaffold characteristics are greatly determined by the inherent characteristics of the scaffold materials. Basically, two main requirements for scaffold material are: biocompatibility (i.e., not to stimulate any unwelcome tissue response to scaffold, and simultaneously to have an appropriate surface chemistry so as to assist cell attachment and proliferation) and biodegradability (i.e., capable of being broken down into nontoxic products after a specific period). TE scaffolds are normally produced from polymers, ceramics or polymer/ceramic composites.

To date, bioceramics such as the calcium phosphate family, including HA, \(\beta\)-TCP and bioactive glasses, have demonstrated good bioactivity and biocompatibility and are widely used as artificial bone matrix and filler material for bone injury repair. Bioceramics suitable for TE scaffolds can be classified into three main groups: bioinert ceramics such as alumina and zirconia; bioactive ceramics such as bioglass, sintered HA (s-HA) and alumina-wollastonite glass ceramic (AWGC); and bioresorbable ceramics such as sintered HA (u-HA), \(\alpha\) or \(\beta\)-TCP, tetracalcium phosphate (TTCP) and octacalcium phosphate (OCP). Bioceramic ceramics are an appropriate choice of bioceramics as reinforcing element where biodegradability is important. Their rates of biodegradation are in the following order: OCP > \(\alpha\)-TCP > \(\beta\)-TCP > u-HA (Yang et al., 2001).

Natural polymers including chitosan, glycosaminoglycan, collagens, starch and chitin have been exploited for regeneration of different tissues such as cartilage, bone, nerves and skin. While naturally occurring biomaterials may most closely simulate the native cellular milieu, the main limitation for their wider application is large batch-to-batch variations upon isolation from biological tissues (Yang et al., 2001). Apart from that, scaffolds made from natural polymers such as collagen and chitin had poor and inadequate mechanical performance. To overcome these limitations of natural polymers, synthetic resorbable polymers such as polyphosphazens, polyanhydrides (PAs), poly (a-hydroxy esters), and polyorthoesters were utilized. Poly(a-hydroxy ester) s and copolyesters of lactic acid and glycolic acid form a significant group of synthetic biodegradable polymers. Polylactic acid (PLA), polyglycolide (PGA), PCL, PAs, polyorthoesters, polydioxanone and copolymers thereof
are biodegradable, synthetic polymers which have been used for years in surgical sutures, and have a long and proven clinical record. Some biodegradable polymers such as PLLA can be used in composite systems with ceramic fillers due to their initial high strength. PLLA and PGA exhibit a high degree of crystallinity and degrade relatively slowly, while copolymers of PLLA and PGA (i.e., PLGA) are amorphous and degrade rapidly (Yang et al., 2001). Table 2.1 shows the properties of some biodegradable polymers.

Scaffolds made from some other biodegradable polymers such as PPF, poly (1,8)octanediol citrate (POC), and poly (glycerol-sebacate) (PGS) have exhibited good results in terms of mechanical properties. Hydrogel can also be used for tissue regeneration scaffolds; it is a biodegradable biomaterial in the form of a colloidal gel in which water is the dispersion medium and it is formed by the cross-linking network of hydrophilic polymer chains (Bartolo and Bidanda, 2008; Nguyen and West, 2002). The structure of hydrogels can be compared with elastin and collagen which form the natural tissue. Hydrogels are normally formulated from a wide range of materials including silicon, cellulose derivatives, poly (vinyl alcohol), poly (ethylene glycol), calcium alginate and the most widely used poly (hydroxyethyl methacrylate) (PHEMA) (Bartolo and Bidanda, 2008).

Apart from the advantages mentioned for polymers, they are ductile and not adequately rigid for some TE applications. Scaffolds with properties closer to natural load bearing tissues can be obtained by combining polymers with bioceramics which are too stiff and brittle by themselves.

Biocompatible metals such as stainless steels, and cobalt- and titanium-based alloys have been employed extensively for different biomedical applications like surgical implants. However, lack of biodegradability and processability are the two main obstacles for biocompatible metals in TE applications. Thus, polymers and polymer/ceramic composites have been considered more by researchers for TE scaffold applications.

Both macro- and micro-structure of scaffolds need to be designed based on the desirable performance and application. Scaffold may have simple or complicated macrostructure depending on the application. Macrostructure would be complex where reconstruction of a damaged organ/tissue of a patient is required on the basis of acquired medical images such as magnetic resonance imaging (MRI) or computed tomography (CT) scans. The main advantage of SFF techniques is that they are able to fabricate TE scaffolds with both predefined macro- and micro-structures.

It has been proved that porosity and pore size of the supporting 3D structures are two important factors which affect regeneration of specific tissues using synthetic substances. Cell attachment and ingrowth are promoted by increasing surface area. Highly porous scaffolds are favourable for improving nutrient diffusion and removing waste products and also for more efficient vascularization. In particular, mass transport control is an important
Table 2.1 Properties of biodegradable polymers suitable for TE scaffolds

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Melting point (°C)</th>
<th>Glass transition temperature (°C)</th>
<th>Degradation time (months)*</th>
<th>Density (g/cm³)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation (%)</th>
<th>Modulus (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA</td>
<td>Amorphous</td>
<td>45–55</td>
<td>Adjustable</td>
<td>1.27–1.34</td>
<td>41.4–55.2</td>
<td>3–10</td>
<td>1.4–2.8</td>
</tr>
<tr>
<td>DL-PLA</td>
<td>Amorphous</td>
<td>55–60</td>
<td>12–16</td>
<td>1.25</td>
<td>27.6–41.4</td>
<td>3–10</td>
<td>1.4–2.8</td>
</tr>
<tr>
<td>L-PLA</td>
<td>173–178</td>
<td>60–65</td>
<td>&gt;24</td>
<td>1.24</td>
<td>55.2–82.7</td>
<td>5–10</td>
<td>2.8–4.2</td>
</tr>
<tr>
<td>PGA</td>
<td>225–230</td>
<td>35–40</td>
<td>6–12</td>
<td>1.53</td>
<td>&gt;68.9</td>
<td>15–20</td>
<td>&gt;6.9</td>
</tr>
<tr>
<td>PCL</td>
<td>58–63</td>
<td>−65</td>
<td>&gt;24</td>
<td>1.11</td>
<td>20.7–34.5</td>
<td>300–500</td>
<td>0.21–0.34</td>
</tr>
</tbody>
</table>

* Time to complete mass loss.

issue for bone TE scaffolds since the high rates of nutrient and oxygen transfer at the surface of the scaffold promote the mineralization of the scaffold surface, further limiting the mass transfer to the interior of the scaffold (Sachlos and Czernuszka, 2003). As a consequence, cells would be able to survive only close to the surface. Using larger pore size in the external areas of the scaffold would not be an effective measure since it may result in degradation of mechanical properties of the bone scaffolds. The minimum pore size of a scaffold is usually defined by the diameter of cells in suspension which differs from one cell type to another. Experiments show optimum pore size of 5 μm for neovascularization, 5–15 μm for fibroblast ingrowth, close to 20 μm for the ingrowth of hepatocytes, 20–125 μm for regeneration of adult mammalian skin, 40–100 μm for osteoid ingrowth and 100–350 μm for regeneration of bone (Yang et al., 2001). For rapid vascularization and survival of transplanted cells fibrovascular tissues need to possess pore sizes greater than 500 μm (Wake et al., 1994). Interconnectivity of pores within the scaffold is another important issue that should be taken into consideration. If the pores are not interconnected within the scaffold, mass transport (that is permeability and diffusion) and cell migration will not happen appropriately, even if the porosity of the scaffold is high.

Techniques in macro-microstructure design for patient-specific scaffold must be able to firstly offer hierarchical porous structures so that the required mechanical function and mass transport are satisfied and, secondly, these structures must be embedded in complicated and arbitrary 3D anatomical shapes. Computational topology design (CTD) is an effective design procedure which can be integrated with SFF for design and fabrication of TE scaffold. CTD-based scaffold design may start with the creation of unit cell libraries that can be assembled to form scaffold architectures. Unit cells libraries may be created by either using approaches based on CAD (Cheah et al., 2004; Fang et al., 2005; Van Cleynenbreugel et al., 2002) or using image-based design approaches (Hollister et al., 2000, 2002; Lin et al., 2004b). Homogenization theory (Hollister and Kikuchi, 1994) can then be exploited to calculate effective properties based on these unit-cell designs. Surpassing effective property calculation from defined microstructures, topology optimization approaches (Lin et al., 2004a; Sigmund, 1994) actually compute new microstructures to attain desired properties. There is the possibility of either optimizing functional elastic properties with a constraint on porosity or maximizing permeability with a constraint on required elastic properties (Hollister, 2006). Scaffold architecture creation within a complex 3D anatomical shape is the final stage of the scaffold design process. 3D anatomic shape is generated from CT or MRI images of the patient. Both CT and MRI produce structured voxel datasets where patient anatomy is defined by density distribution. These datasets can be used in the design process either by converting the voxel anatomic data into solid geometric models for
use in CAD (Cheah et al., 2004; Fang et al., 2005; Van Cleynenbreugel et al., 2002) or by directly using voxel database structures in image-based methods (Hollister et al., 2000, 2002). Finally, Boolean techniques are used to intersect the defined anatomic defected shape with the microstructure design database, resulting in the final patient-specific scaffold design (Hollister, 2006).

2.5.2 Scaffold fabrication using extrusion freeforming

Various fabrication methods including traditional chemical engineering methods and advanced SFF techniques are currently used for construction of TE scaffolds. Traditional techniques to fabricate TE scaffolds include: solvent casting/salt leaching, phase separation, foaming and textile meshes. These techniques have several limitations as they cannot usually control pore size, pore geometry or spatial distribution of pores properly. In contrast, SFF advanced techniques can simply control the internal and external structure of scaffolds and overcome some intrinsic limitations of conventional methods such as shape restrictions, manual intervention and inconsistent and inflexible processing procedures. In order to take advantage of these breakthroughs, there has been a trend in recent years towards fabrication of TE scaffolds using SFF processes directly (building the final scaffold) or indirectly (building a negative scaffold for use as a mould). In particular, extrusion-based SFF systems have been widely investigated for making TE scaffolds due to their ability to process different biomaterials, and to manufacture scaffolds in a cell-friendly environment, their high reproducibility and flexibility, and their simple process control in comparison with other SFF techniques. As mentioned earlier, extrusion-based freeforming techniques are classified into two main groups: processes with and without material melting. Figure 2.12 illustrates the working principle of the key extrusion-based methods schematically. In the following section, the principles of each process are described and the main issues associated with each process will be highlighted.

Extrusion freeforming with material melting

FDM is the first SFF system based on extrusion of polymer melts. Thermoplastic materials in the form of filament are used as feedstock and a pinch roller feed mechanism is used to push the filament into the liquefier, with subsequent extrusion from a computer-controlled nozzle. By repeating the extrusion process the final part is fabricated layer by layer. There is demand for precise temperature control system to achieve desirable accuracy. Several modified FDM systems have been developed for fabrication of 3D scaffolds with micron size pores and filaments. Recent specialized FDM systems for 3D scaffold mostly use a screw feed instead of a pinch roller feed mechanism to enhance extrusion accuracy.
2.12 Schematic illustration of different extrusion-based systems, including processes with and without material melting: (a) FDM process (Zein et al., 2002); (b) 3D fibre deposition process (Woodfield et al., 2004); (c) PEM process (Xiong et al., 2001); (d) PED process (Wang et al., 2004); (e) LDM process (Zhuo et al., 2002); (f) 3D bioplotting process (Landers et al., 2002); (g) pressure-assisted writing processes such as PAM and direct-write assembly techniques (Vozzi et al., 2002); (h) paste extrusion techniques such as robocasting and solvent-based extrusion freeforming techniques (Miranda et al., 2008). It should be noted that robocasting process can be carried out under proper coagulation reservoirs such as oil bath or cooled bath containing dry ice, whereas solvent-based extrusion freeforming deposits biomaterials at room temperature.

(Continued)
FDM has been used successfully to produce scaffolds in PCL, polypropylene (PP)/TCP, PCL/HA, PCL/TCP with a resolution of 250 μm. Hutmacher and his group (Zein et al., 2002) have extensively investigated the process parameters for PCL and they fabricated several composites including PCL/HA and PCL/TCP scaffolds using FDM. Bone TE scaffolds produced from polymer and calcium phosphate (CaP) using FDM have exhibited good mechanical and degradation properties, improved cell seeding and enhanced incorporation and immobilization of growth factors. As for mechanical properties, the existence of the CaP phase brings about higher structural strength and the polymer phase provides plasticity and toughness to the scaffold. Kalita et al. (2003) produced controlled porosity polymer-ceramic composite PP/TCP scaffolds, with 3D interconnectivity designed to promote a richer supply of blood, oxygen and nutrients for healthy ingrowth of bone cells. Controlled porosity alumina and β-TCP ceramic scaffolds with pore sizes in the range 300–500 μm and pore volumes of 25%–45% have been produced using the indirect fused deposition process (Bose et al., 2003). Safari and his group (Allahverdi et al., 2001) produced a hybrid scaffold from alumina and wax (as the support structure) directly using multi-nozzle fused deposition of ceramics (FDC). Highly porous PLGA scaffolds for cartilage TE were fabricated by Hung-Jen et al. (2009) using FDM and were further modified by type II collagen. Tellis et al. (2008) used micro computed tomography (CT) to create biomimetic polybutylene terephthalate (PBT) trabecular scaffolds using an FDM process.

Two major limitations of FDM processes are the need to use filamentary materials as feedstock and the high heat effect on raw biomaterial. In particular, preparing filamentary feedstock makes it difficult/time consuming to process new biomaterial. To overcome this problem, some alternative processes with new configurations such as 3D fibre deposition, PED, PEM and MJS processes have been proposed.
In 2004, Woodfield et al. (2004) developed an extrusion-based system called 3D fibre deposition with the aim of extruding highly viscous polymer. The technique allowed them to make scaffolds by accurately controlling the deposition of molten co-polymer fibres from a pressure-driven syringe onto a computer controlled xyz table. As seen in Fig. 2.12b, the 3D fibre deposition consisted of five main components: (1) a thermostatically controlled heating jacket; (2) a molten copolymer dispensing unit consisting of a syringe and nozzle; (3) a force controlled plunger to regulate flow of molten co-polymer (4); a stepper motor-driven xyz table; and (5) a positional control unit consisting of stepper motor drivers linked to a personal computer containing software for generating fibre deposition paths. Woodfield and co-workers produced 3D poly(ethylene glycol)-terephthalate/poly(butylene terephthalate) (PEGT/PBT) block co-polymer scaffolds (Woodfield et al., 2004) and polyethyleneoxide terephthalate (PEOT) and PBT scaffolds (Moroni et al., 2006) with a 100% interconnecting pore network.

The PED process is another material melting extrusion-based process, developed by researchers at Drexel University (Wang et al., 2004). The working principle of PED is similar to FDM barring that material in the form of pellets or granules is liquefied in a chamber and a rotating screw (mini-extruder) forces the material through the nozzle. Shor et al. (2007) used this method to build PCL and PCL/HA 3D scaffolds with uniform pore size of 250 μm. The test results reported for PCL scaffolds produced by PED proved the structural integrity, controlled pore size, pore interconnectivity, favourable mechanical properties and basic biocompatibility (Shor et al., 2009). Hoque et al. (2009) developed a desktop robot-based rapid prototyping (DRBRP) system with the ability to process a wide range of synthetic polymers in the form of pellet, lump or powder to build 3D scaffolds. Their biocompatibility tests using rabbit smooth muscle cells proved excellent performance of fabricated scaffolds in terms of cell adhesion and tissue formation.

Xiong et al. (2001) have developed a PEM process in which compressed air is used instead of a piston or rotating screw to push the melted biomaterial through the deposition nozzle. With the computer-controlled digital valve upon the deposition nozzle, the switch response speed is high and filaments can be deposited with sufficient accuracy onto the substrate. Xiong and his group fabricated different porous PLLA and PLLA/TCP bone tissue engineering scaffolds with different properties and with controlled architecture and geometry through this PEM process.

The MJS process was developed at the Fraunhofer-Gessellschaft research centre to produce high density metallic or ceramic parts using low melting point alloys or a powder-binder mixture (Greulich et al., 1995). Heated paste is pushed out through a nozzle and deposited onto a computer-controlled build table. The feedstock is normally supplied as powder, pellet or bar and
the extrusion temperature of the molten material can reach up to 200°C. Powder-binder feedstock is heated in a process chamber above the melting point of the binder, and thus only the binder is liquefied during the process. A piston is used to push out the low viscous flow through the nozzle and the material is deposited layer by layer. MJS was used to build 3D scaffolds made of poly (D, L-lactide) (PDLLA) for bone and cartilage tissue engineering. The scaffold pore size was found to be in the range 300–400 μm and the structure supported ingrowth of human bone tissue (Koch et al., 1998).

The Polytechnic Institute of Leiria developed a variation of FDM called BioExtruder for producing PCL scaffolds (Domingos et al., 2009; 2012). BioExtruder comprises two different deposition systems: one rotational system for multi-material deposition acted by a pneumatic mechanism and another one for a single material deposition that uses a screw to assist the deposition process.

**Extrusion freeforming without material melting**

New configurations for melt extrusion could open up the possibility for the use of a wider range of biomaterials, making the extrusion-based systems a more versatile and realizable alternative manufacturing process for composite scaffold materials. But limitations remain in terms of the high heat effect on raw biomaterial. Thus, researchers have made attempts to develop new configurations to process biomaterials without melting that can better preserve the bioactivities of the scaffold materials.

The PAM process is a technique developed by Vozzi and co-workers, (2002) which resembles FDM without the need for heating. PAM uses a pneumatic driven microsyringe to deposit biomaterial on a substrate. Material viscosity, deposition speed, tip diameter and the applied pressure correlate with the final deposited strand dimensions (Vozzi et al., 2003). Polymeric scaffolds with different polymer compositions such as PCL, PLLA, PLGA, PCL/PLLA, gelatin and alginate hydrogel scaffolds with three different geometries – square grids, hexagonal grids and octagonal grids were produced (Mariani et al., 2006; Tirella et al., 2008, 2009; Vozzi and Ahluwalia, 2007; Vozzi et al., 2004). Apart from TE scaffolds, PAM was used to deposit a polyurethane dielectric layer and a carbon black electrode layer above it (Tartarisco et al., 2009). Vozzi and his group used a modified system called piston assisted microsyringe (PAM2) for microfabrication of viscous, sol–gel or gelled inks (e.g., alginate solutions at different concentrations). PAM2 uses a stepper motor instead of compressed air to move the syringe plunger with a controlled speed (Tirella et al., 2012). PAM2 also has a temperature controlled syringe (TCS) module to control the temperature of processed materials using an aluminium jacket.
The key feature of the LDM process proposed by Xiong et al. (2002) is that it is a non-heating liquefying process. In it, material slurries are fed into the material supply that is connected to a screw pump nozzle using a soft pipe and the fabrication process is accomplished in a low temperature environment below 0°C in the freezer. The layer of deposited materials is frozen on the platform. After the forming process, the frozen scaffolds formed by the LDM system need to be freeze-dried for rather a long time (~38 h) to remove the solvent. The bone scaffolds made by this LDM system have good biocompatibility and bone conductive property as a molecular scaffold for bone morphogenetic protein in the implantation experiments of repairing segment defects of rabbit radius (Yongnian et al., 2003). Biomolecules can be applied in the LDM process to directly fabricate a bioactive scaffold. Incorporating multiple nozzles with different designs into the LDM technique gave rise to multinozzle low-temperature deposition and manufacturing (M-LDM) and multinozzle deposition manufacturing (MDM) (Liu et al., 2009a, 2009b). The M-LDM system is proposed for fabricating scaffolds with heterogeneous materials and gradient hierarchical porous structures by the incorporation of more jetting nozzles into the system. The LDM process has been used to build multi-material (Liu et al., 2009a), and different hydrogel scaffolds (Li et al., 2009a, b). Cong Bang et al. (2008) developed a special LDM system based on rapid freeze prototyping (RFP) to produce scaffolds from chitosan solution.

3D bioplotting is a technique that was first developed by Landers and Mulhaupt (2000) at Freiburger group to produce scaffolds for soft tissue engineering purposes, and simplifying hydrogel manufacture. 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. It is possible to perform either discontinuous dispensing of microdots or continuous dispensing of fine filaments. 3D bioplotting can process thermally sensitive biocomponents and cells since heating is not applied. Curing reactions can be performed by plotting in a co-reactive medium or by two-component dispensing using mixing nozzles. The filament thickness can be adjusted by varying the viscosity of the plotting solution, nozzle diameter and the applied pressure (Billiet et al., 2012). Further surface treatment is normally applied to the scaffolds produced by 3D bioplotting as they mostly have smooth surfaces that are undesirable in terms of cell attachment. Geun Hyung and Joon Gon (2009) used a piezoelectric transducer (PZT) generating vibrations during plotting to make PCL scaffolds with a rough surface. Maher et al. (2009) developed a device based on bioplotting with the ability to heat the plotting materials and produced TE scaffolds using a variety of
materials including poly (ethylene glycol) (PEG), gelatin, alginic acid and agarose at various concentrations and viscosities.

In comparison with other extrusion-based SFF processes, 3D bioplotting can process a remarkably wide variety of different biomaterials, including polymer melts, thermoset resins, polymer solutions and pastes with high filler contents, and bioactive polymers such as proteins. The plotting of biomaterials such as melts of PLA, PLGA, PHBV biodegradable thermoplastic, PCL, poly (butylene terephthalate-block oligoethylene oxide), biopolymer solutions of agar and gelatin (Landers et al., 2002), natural polymers such as collagen and reactive biosystems involving fibrin formation and polyelectrolyte complexation are all possible. In particular, the processing of materials with low viscosities benefits from buoyancy compensation (Pfister et al., 2004). The work of the Freiburger group led to the commercialization of the first 3D bioplotting system by EnvisionTec GmbH (www.envisiontec.com) to meet the demand for 3D scaffolds with well-defined external and internal structures in tissue engineering and controlled drug release. The 3D-Bioplotter™ has the capacity to fabricate scaffolds using the widest range of materials from soft hydrogels and polymer melts to hard ceramics and metals.

Recently, Schuurman et al. (2011) used a hybrid bioplotting approach for fabrication of solid biodegradable material (polymers, ceramics) with cell-laden hydrogels that could combine favourable mechanical properties with cells positioned in defined locations at high densities. The resulting mechanical properties of the scaffolds were significantly improved and could be tailored within the same range as those of native tissues. Moreover, the approach allows the use of multiple hydrogels, and can thus build constructs containing multiple cell types or bioactive factors. Furthermore, since the hydrogel is supported by the thermoplastic material, a broader range of hydrogel types, concentrations and cross-link densities can be used compared to the deposition of hydrogels alone, thereby improving the conditions for encapsulated cells to proliferate and deposit new matrix (Melchels et al., 2012).

Khalil et al. (2005) developed a special multinozzle bioplotter which was capable of extruding biopolymer solutions and living cells for freeform construction of 3D tissue scaffolds. The deposition is not into plotting media but the process is biocompatible and occurs at room temperature and low pressures to reduce damage to cells. The system was capable of, simultaneously with scaffold construction, depositing a controlled amount of cells, growth factors or other bioactive compounds with precise spatial position to form complex cell-seeded tissue constructs. They fabricated some scaffolds based on sodium alginate solutions and PCL.

Ang et al. (2002) set up a special robotic bioplotting device called rapid prototyping robot dispensing (RPBOD) for the design and fabrication of
chitosan-HA scaffolds. Their system consists of a computer-guided desktop robot and a one-component pneumatic dispenser. Mixtures of sodium hydroxide solution and ethanol at different ratios were used as plotting medium to produce chitosan-HA scaffolds.

Further, the RPBOD system was improved to include a new manufacturing method called the dual dispensing system as, besides the pneumatic dispenser, a mechanical dispenser driven by a stepper motor was set up to deposit plotting medium (NaOH). The dual dispensing method overcomes the high sensitivity to material concentration compared with the method of dispensing plotting materials into a fluid medium, as precipitation occurs when the dispensing material and the coagulant medium merge on the base or on the previous layer. There is therefore no precipitated lump forming at the nozzle and no movement of the fluid medium to affect the shape of the precipitated strands of the scaffold. The chitosan scaffolds built by researchers at National University of Singapore using RPBOD exhibit excellent uniformity, interconnectivity, sufficient strength, good reproducibility and calibration (Li et al., 2005).

A variety of extrusion-based techniques has also been developed for processing ceramics. 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. The slurry is deposited layer by layer from a syringe using constant displacement at a controlled rate. Upon deposition, robocasting relies on a small amount of drying to induce rheological transition of the slurry. The slurry changes from a flowable pseudo-plastic state to a solid-like dilatant mass. This transition gives each layer the strength necessary to support subsequent layers of freshly deposited slurry. Robocasting is a binderless process with low toxicity in which drying is necessary to build 3D parts. The concept of robocasting relies essentially on the rheology of the slurry and also on the partial drying of the deposited layers. In order to make good parts, high solids loadings are necessary, so powder surface chemistry and interparticle forces must be controlled. In addition to the aforementioned factors, the parameters associated with the freeform process, including table speeds, deposition rate and nozzle size, should be controlled appropriately. Miranda et al. (2006; 2008) used a robocasting process to produce β-TCP scaffolds with designed 3D geometry and mesoscale porosity using concentrated β-TCP inks with appropriate viscoelastic properties. The deposition was done in a non-wetting oil bath to prevent non-uniform drying during assembly.

Direct-write assembly is an extrusion-based system developed by Lewis and co-workers (Smay et al., 2002) whereby a wide range of inks can be patterned in both planar and 3D shapes with feature sizes as fine as 250 nm. 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. In this latter process, compressed air is employed to push inks with controlled rheological properties through an individual nozzle (diameter ranging from 1 to 500 μm). The key components of a direct-write assembly system are: compressed air supply, nozzle, three-axis translation stage and optical microscope for real-time monitoring. Direct-write assembly deposits inks on substrates at room temperature or a proper coagulation reservoir using a controlled-printing speed and pressure which depend on ink rheology and nozzle diameter. Due to viscoelastic ink characteristics, direct-write assembly enables self-supporting and spanning features. Ink rheology strongly depends on solid loading for nanoparticle inks so that viscosity decreases by decreasing solid loading. Concentrated inks with solid loadings of 70–85 wt.% are normally required for printing planar and spanning filaments. Using low viscosity inks (i.e., dilute inks) results in a significant lateral spreading during printing.

A wide range of inks including colloidal suspensions and gels, nanoparticle-filled inks, polymer melts, fugitive organic inks, hydrogels, sol–gel and polyelectrolyte inks have been processed using direct-write assembly. Lewis and co-workers have achieved minimum feature sizes ranging from 250 nm for sol-gel inks to 200 μm for ceramic colloidal inks. Writing with some inks such as polyelectrolyte inks needs to be performed into a reservoir-induced coagulation to enable 3D printing, whereas some other inks such as sol-gel inks can be directly printed in air providing excellent control over the deposition process (e.g., the ink flow can be started/stopped repeatedly during assembly).

In recent years, Lewis and co-workers have focused on extending direct-write assembly to biomedical applications. Using biocompatible inks they printed different 3D scaffolds and microvascular networks for tissue engineering and cell culture. Different 3D HA scaffolds with 250 μm road width (Michna et al., 2005; Simon et al., 2007), and 3D scaffolds composed of a gradient array of silk/HA filaments of 200 μm size were fabricated by direct-write assembly (Sun et al., 2012). The 3D silk/HA scaffolds were used to support the growth of co-cultures of human bone marrow-derived mesenchymal stem cells (hMSCs) and human mammary microvascular endothelial cells (hMMECs) to assess in vitro formation of bone-like tissue. 3D microperiodic scaffolds of regenerated silk fibroin have been fabricated using direct-write assembly for tissue engineering (Ghosh et al., 2008). Biocompatible silk optical waveguides as fine as 5 μm were produced by direct-write assembly of a concentrated silk fibroin ink through a micronozzle into a methanol-rich coagulation reservoir (Parker et al., 2009). 3D microperiodic hydrogel scaffolds composed of 1 μm (Barry et al., 2009) and 10 μm (Shepherd et al., 2011) filaments
were produced for guided cell growth by direct writing through a gold-coated deposition micronozzle of a PHEMA-based ink that is simultaneously photopolymerized via UV illumination.

Solvent-based extrusion freeforming is another technique developed by our group to produce bioceramic scaffolds (Grida and Evans, 2003). In this process, continuous flow of materials in the form of paste or particulate slurries is dispensed onto the surface using a 3D motion system incorporated with the nozzle. Solvent-based extrusion freeforming is a relatively simple process in which phase change is based on solvent evaporation. Paste with a high yield strength is prepared by blending polymer, ceramic and a solvent in specific ratios. Defects such as dilatancy, drying cracks and surface fracture which happen in water-based extrusion systems (Yang et al., 2008c) can be eliminated by appropriate adjustment of polymer content. Low solvent and high ceramic contents result in low drying and sintering shrinkages, respectively. Typical solvent-based extrusion freeforming equipment is shown in Fig. 2.13a and the schematic arrangement is described in Fig. 2.13b. As seen in Fig. 2.13a, there are four axes, including X, Y, Z and an extrusion drive. The stainless steel/glass syringe is mounted in the Z-axis and the sample substrate is placed on the X-Y table. The extrusion pressure can be measured by a load cell which is mounted on the extrusion axis. The control program, compiled by Labview Software (National Instruments, US), controls four motors for the movement of X-, Y- and Z-axes and extrusion. The overall solvent-based extrusion freeforming process steps are: (1) preparation of paste, (2) deposition of fine filaments and (3) post-processing including drying, debinding and sintering. The paste is normally prepared using an ultrasonic probe for dispersion of powder, drying to increase viscosity and limited vacuum de-airing. Thermoplastic binder, polyvinylbutyral (PVB) and plasticizer, PEG (MWt = 600), in the ratio of 75 wt.% PVB and 25 wt.% PEG, are fully dissolved in the solvent, propan-2-ol and then the desired bioceramic is added to the solution and finally is stirred (for at least 2 h) to achieve a well-dispersed paste.

A range of bioceramic scaffolds have been fabricated by our group with different compositions in the HA/β-TCP (different HA/β-TCP ratios) and sintered from 1100°C to 1300°C in steps of 50°C. Scaffolds with different porosities and pore sizes were produced, with raster width down to 60 μm and interconnected pores with interstices from 50 to 500 μm (Yang et al., 2008a, b, c). Other ceramic pastes such as alumina, alumina/silica, zirconia and alumina/graphite have been used successfully for fabrication of 3D lattice structures with fine filaments (Xuesong et al., 2009b, 2010). In addition to bioceramics, 3D carbon scaffolds have been produced using two different paste compositions with different polymers in the paste (Lu et al., 2012). Figure 2.14 shows a sample HA scaffold with filament diameter 70 μm produced by solvent-based extrusion freeforming.
Bioceramic scaffolds produced by solvent-based extrusion freeforming have the finest filament diameter amongst extrusion-based SFF techniques such as direct-write assembly or robocasting. However, producing bioceramic scaffolds with fine filaments (less than 100 μm) is still a challenging issue. A set of experiments was conducted to define the most significant factors that should be taken into consideration in order to reach fine filaments. Equipment accuracy (in particular levelling error) and appropriate adjustment of X-Y table velocity and nozzle path according to extrusion ram velocity have been determined as the most important factors for fine filament extrusion freeforming. The levelling error determines how large a sample can be fabricated. The relationship between X-Y table velocity
and extrusion ram velocity under steady state extrusion conditions follows Equation [2.1]:

\[ V_{\text{ram}} = \left( \frac{D_{\text{nozzle}}}{D_{\text{barrel}}} \right)^2 \]

where, \( V_{\text{ram}} \) is extrusion ram velocity, \( V_{\text{paste}} \) is paste extrude velocity, \( D_{\text{barrel}} \) is syringe barrel diameter and \( D_{\text{nozzle}} \) the nozzle diameter. Based on Equation [2.1], the X-Y table velocity can be a thousand times higher than the extrusion ram velocity for extrusion of very fine filaments (smaller nozzle diameter), depending on the ratio of syringe barrel diameter to nozzle diameter. Hence, some control difficulties are introduced in order to make the X-Y table and extrusion work in harmony. Therefore, the ratio of barrel to nozzle diameter should be selected to adjust these two velocities (Xuesong et al., 2009b). In short, X-Y table velocity should match the extrusion ram velocity and barrel diameter should be compatible with the nozzle diameter. Overfill and underfill of filament deposition can happen in acceleration and deceleration paths where extrusion is not in a steady state. Moreover, it was observed that air bubbles and particle agglomerates are two of the most common defects in fine filaments which are often produced in the paste preparation stage. The solvent content of paste is the most important
parameter which determines the ability of the filament to span and retain planned height. Leakage paths and die swell can occur during extrusion and both perturb the ideal relationship between ram velocity and extrusion rate, hence hindering the control system. Low ram velocity can be selected to reduce die swell.

The extrusion pressure $P$ for paste extrusion described by Benbow and Bridgewater (1993) and expressed by Equation [2.2]:

$$P = f + P_1 + P_2 = f + 2(\sigma_0 + \alpha V^m)\ln\left[\frac{D_0}{D}\right] + 4(\tau_0 + \beta V^n)\left[\frac{L}{D}\right]$$  [22]

where $f$ is the friction between the plunger and the syringe wall; $P_1$ is the pressure drop in the die entry; $P_2$ is the pressure drop in the die land; $V$ is the paste velocity in the die land; $\sigma_0$ is the yield stress extrapolated to zero velocity; $\alpha$ is a factor characterizing the effect of velocity; $\tau_0$ is the initial wall stress; $\beta$ is the wall velocity factor which accounts for the velocity-dependence of the wall shear stress; $m$ and $n$ are exponents for taking account of non-linear behaviour of the paste; $D_0$ is the diameter of the barrel; $D$ is the diameter of the die land; and $L$ is the length of the die land (see Fig. 2.15).

According to Equation [2.2], increasing the die land length ($L$) results in a remarkable increase of extrusion pressure so that extrusion pressure is slightly higher in processes such as PAM or direct-write assembly than solvent-based extrusion freeforming as they use needle like nozzles. The authors used nozzles with small die land length (~1 mm) to decrease the required extrusion pressure as much as possible. According to Equation [2.2], decreasing nozzle diameter results in increased extrusion pressure. The authors could decrease nozzle diameter and achieve fine filaments of 60 $\mu$m but decreasing nozzle diameter is not a matter to be taken lightly as it causes nozzle clogging.

From what we have discussed above, extrusion-based SFF processes can be employed as an efficient standard tool in tissue engineering. However, choosing the right extrusion-based process requires careful evaluation of the capabilities and limitations of each process. Table 2.2 provides a comparison between the key extrusion-based SFF techniques in terms of resolution, materials and their characteristics.

Scaffold-based tissue engineering is a proven approach in regenerative medicine but is still subject to some limitations and challenges including: (1) complications posed by host acceptance (immunogenicity, inflammatory response, mechanical mismatch); and (2) problems related to cell cultures (e.g., cell density, multiple cell types, specific localization) (Billiet et al., 2012). Cell-based printing techniques have been intensively investigated in recent years and many innovative approaches such as organ bioprinting (Mironov et al., 2007), laser writing of cells (Schiele et al., 2009), bio-electrospraying
(Jayasinghe, 2007) and biological laser printing (BioLP) (Barron et al., 2004) have surfaced to complement limitations in scaffold-based TE. Billiet and co-workers (2012) define organ bioprinting as ‘the engineering of 3D living structures supported by the self-assembly/organizing capabilities of cells delivered through the application of SFF techniques based on laser, inkjet, or extrusion freeforming technologies’.

Inkjet and extrusion-based systems are utilized in a similar way to direct bioprinting: balls or continuous flows of bioinks are deposited in well-defined topological patterns into biopaper layers. The bioink building blocks typically have a spherical or cylindrical shape, and consist of single or multiple cell types. In a post-processing step, the construct is transferred to a bioreactor and the bioink spheres are fused. The biopaper, an inert and biocompatible hydrogel, can be removed after construction in post-processing.
<table>
<thead>
<tr>
<th>Technique</th>
<th>Lateral resolution (μm)</th>
<th>Materials</th>
<th>Strengths</th>
<th>Drawbacks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDM</td>
<td>250</td>
<td>PCL, PP-TCP, PCL-HA, PCL-TCP, PLGA, polybutylene terephthalate (PBT), etc.</td>
<td>Good mechanical strength, versatile in lay-down pattern design, no trapped particles or solvents</td>
<td>High temperature, need to produce filament material, rigid filament, limited material range, difficult to prepare structures with microscale porosity</td>
<td>Bose et al., 2003; Hung-Jen et al., 2009; Kalita et al., 2003; Tellis et al., 2008; Zein et al., 2002</td>
</tr>
<tr>
<td>RPBOD/dual dispensing</td>
<td>400</td>
<td>Chitosan-HA; chitosan</td>
<td>Enhanced range of materials can be used, can incorporate biomolecules</td>
<td>Low mechanical strength, precise control properties of material and medium, requires freeze drying</td>
<td>Ang et al., 2002; Li et al., 2005</td>
</tr>
<tr>
<td>PAM</td>
<td>5–10</td>
<td>PCL, PCL-PLLA, PLGA, PLLA, polyurethane elastomer (Polytek 74–20), alginate, gelatin and viscous inks (using PAM2)</td>
<td>Enhanced range of materials can be used, can incorporate biomolecules, high resolution, not subject to heat, can be used for multilayers</td>
<td>Small nozzle inhibits incorporation of particle, narrow range of printable viscosities, solvent is used, highly water-soluble materials cannot be used</td>
<td>Mariani et al., 2006; Tirella et al., 2009; Vozzi and Ahluwalia, 2007; Vozzi et al., 2004</td>
</tr>
<tr>
<td>Robocasting</td>
<td>100</td>
<td>Ceramic and organic inks</td>
<td>Enhanced range of materials can be used, high ceramic content, multi-material scaffold is possible</td>
<td>Precise control of ink properties is crucial</td>
<td>Cesarano, 1999; Miranda et al., 2008; 2006</td>
</tr>
<tr>
<td>PED</td>
<td>250</td>
<td>PCL, PCL-HA</td>
<td>Input material in pellet form</td>
<td>High temperature, rigid filament</td>
<td>Shor et al., 2009; Shor et al., 2007; Wang et al., 2004</td>
</tr>
<tr>
<td>-------</td>
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</tr>
<tr>
<td>3D bioplotting 45 (hydrogel)-250</td>
<td>Bone regeneration and drug release applications: HA, titanium, TCP, PCL, PLGA, PLLA</td>
<td>Remarkably wide variety of different materials, can incorporate biomolecules, use of hydrogel materials (agar, gelatin, etc.)</td>
<td>Low mechanical strength, smooth surface which is not desired for appropriate cell attachment, low accuracy, slow processing, precise control of properties of plotting material and medium is required</td>
<td>Landers et al., 2002; Landers and Mulhaupt, 2000; Maher et al., 2009; Pfister et al., 2004</td>
<td></td>
</tr>
<tr>
<td>LDM/MDM/M-LDM 300</td>
<td>PLLA-TCP, PLGA, collagen, PLGA-collagen, chitosan, gelatin, alginate</td>
<td>Input material in grain form, preserve bioactivities of scaffold materials because of its non-heating liquefying processing of materials, can incorporate biomolecules</td>
<td>Solvent is used, requires freeze drying</td>
<td>Li et al., 2009a; Liu et al., 2009b; Yongnian et al., 2003</td>
<td></td>
</tr>
<tr>
<td>PEM 200–500</td>
<td>PLLA; PLLA-TCP</td>
<td>Input material is grains</td>
<td>High temperature, rigid filament</td>
<td>Xiong et al., 2001</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
Direct write assembly

250 nm for sol–gel inks
1–20 μm for nanoparticle inks, hydrogel, and polyelectrolyte inks
200–250 for ceramic inks such as HA; silk-HA

Ceramic inks; fugitive organic inks; nanoparticle inks; polymer inks; sol–gel inks

A wide range of materials, very high resolution, non-heating process,

Precise control of ink properties is crucial

Lewis and Gratson, 2004; Shepherd et al., 2011; Sun et al., 2012

3D fibre deposition

250

Poly (ethylene glycol)-terephthalate/polybutylene terephthalate (PEGT-PBT), polyethyleneoxide terephthalate (PEOT) and polybutylene-terephthalate (PBT)

Input material in pellet form, preparation time is reduced

High temperature, rigid filament, difficult to prepare structures with microporosity

Moroni et al., 2006; Woodfield et al., 2004

Solvent-based extrusion freeforming

80

HA, HA-β-TCP, zirconia, alumina, alumina/silica

Simple process yet high resolution, low sintering shrinkage, controlled microporosity of filaments

Precise control of ink properties is crucial

Xuesong et al., 2009b, 2010, Yang et al., 2008a, 2008b, 2008c
step (Billiet et al., 2012). Several extrusion-based systems such as the 3D bioplotter described earlier can be used as a bio-printer, if sterile conditions can be acquired. However, it should be noted that, technologically, bioprinting using SFF techniques is still in its infancy. Different living structures have been produced using hydrogel structures containing viable cells, but the designs have been simple and isotropic, and mechanical properties were not satisfactory (Melchels et al., 2012). Figure 2.16 depicts some examples for which extrusion-based systems have been used in scaffold-based and scaffold-free tissue engineering.

2.6 Dry powder printing

Recently a promising printing method was developed by our research group to print dry powders precisely with the aims of metering, dispensing and multi-material solid freeforming. Metering and dispensing of small amounts of dry powder is an important demand in a wide range of processes and pharmaceutical industries. A variety of powders with different sizes/shapes need to be weighed out and dispensed in pharmaceutical industries for traditional solid-phase organic synthesis and routine analysis for large compound libraries (Islam and Gladki, 2008). In many cases, powders are metered in interim dose forms (within the range from hundreds of micrograms to tens of milligrams) in the combinatorial chemistry of drug development (Morissette et al., 2004). However, manual weighing is often boring, time-consuming and the accuracy is insufficient. Dry powder printing is an efficient alternative to save time and increase the precision of metering and dispensing.

Furthermore, dry powder printing can be incorporated with powder-based SFF techniques such as SLS and 3D printing to bring new possibilities and complement their current challenges. Powder-based processes such as 3D printing and SLS have little further potential for enhancing the resolution for micromanufacturing applications: as the powder size gets increasingly smaller powder handling (recoating) becomes impossible. Moreover, producing true multi-material parts is still a challenge for powder-based SFF systems. Powder-based systems are currently able to build multi-material 3D parts in which material can change only in the vertical direction. Dry powder dispensing systems (especially ultrasonic nozzle dispensing systems) have demonstrated their great ability in precise placement of fine powders (Lu et al., 2007; Yang and Evans, 2007). It is believed that employing a selective dry powder dispensing mechanism incorporated with powder-based systems would be an efficient way to solve the problem of fine powder handling as well as enhancing the capacity to produce multi-material parts with lateral material change. In this way, a higher level of material deposition control could be obtained which is very attractive.
2.16 (a) Schematic illustration of direct-write assembly of a hydrogel-based ink through a gold-coated deposition micronozzle that is simultaneously photopolymerized via UV illumination. (b) SEM micrographs of 3D PHEMA hydrogel scaffold with 10 μm filament and 30 μm pitch produced using direct-write assembly (Shepherd et al., 2011). (c) Multi-material scaffold (poly(lactic-co-glycolic acid), PLGA-collagen) fabricated via M-LDM (Liu et al., 2009a). (d) Schematic illustration of bioprinting tubular structures using extrusion-based freeforming, layer-by-layer deposition of agarose hydrogel (light grey) cylinders and multicellular pig SMC cylinders (dark grey). (e) The printed construct. (f) Engineered pig SMC tubes of different diameters (left: 2.5 mm OD; right: 1.5 mm OD) resulted after 3 days of post-printed fusion and hydrogel removal. Agarose is not remodelled by the cells and can easily be removed after fusion of the bioink (Lee et al., 2010). (g) Fabrication of solid biodegradable materials with cell-laden hydrogels: schematic illustration of a hybrid bioprinting process including alternating steps of printing biodegradable polymer and cell-laden hydrogel. (h) Layering of the dye-containing alginate results in specific confinement of the printed hydrogels (Schuurman et al., 2011).
and a highly important issue in TE scaffold fabrication. Moreover, it is possible to incorporate growth factors and fabricate controlled-release scaffolds. Ongoing works in our lab are focussing on incorporating dry powder printing with SLS in order to extend the approach to the areas of multi-material and micromanufacturing. Figure 2.17 depicts an example of our preliminary results in high resolution dry powder printing for electronics applications. Dry powder printing enabled us to print lines as fine as ~70 μm. The results prove that high resolution, true 3D multi-material parts can be produced efficiently using the proposed approach.

By application of an acoustic energy driven system containing a glass capillary as funnel and a delicate computer control system, the vibrations from a piezoelectric disc can precisely initiate and halt the flow of powder from a fine nozzle. Once the ultrasonic vibration is switched off, arrest of powder flow is brought about by the formation of domes in the capillary due to wall–particle and particle–particle friction. The powder flow rate can be adjusted by varying the frequency and amplitude of the vibration (Yang and Evans, 2004, 2005). In the following section, processing of nanobiomaterials using dry powder printer are discussed in more detail.

Figure 2.18 shows the experimental device set up for printing different nanobiomaterial dry powders. The device includes a computer, an analogue waveform generator (National Instruments Corporation Ltd., Berkshire, UK), a power amplifier (PB58A, Apex Co., USA), a piezoelectric ring (SPZT-4 A3544C-W, MPI Co., Switzerland), a glass nozzle (made from a capillary tube), a purpose-built water vessel and a microbalance (2100 mg ± 0.1 μg, Sartorius AG, Germany). The system generates a voltage signal, which can be varied by different waveforms (e.g., square, sine, triangle and sawtooth), frequencies and amplitudes (Shoufeng and Evans, 2004; Xuesong et al., 2006). The PZT excited by the high-frequency signal (>20 kHz) transmits the vibration through water to the capillary. The inner diameter of the water tank is 40 mm, with an inserted feed tube made of a 10 mm (inner diameter) glass capillary. The upper section functions as a hopper for the powder sample. The piezoelectric ceramic ring was attached to the bottom of the glass tank with an adhesive commonly used in ultrasonic cleaning tank construction (9340 GRAY Hysol Epoxi-Patch Structural Adhesive, DEXTER Co., Seabrook, USA). The microbalance is employed to verify and record the dose mass.

Different nanobio dry powders have been processed and characterized based on particle size, density, shape and angle of repose (Table 2.3). Angle of repose is tested as a relative measure of friction and cohesiveness of the powders. Generally a powder with an angle of repose greater than about 40°
is classified as cohesive and non-free flowing, which is difficult to dispense in conventional dry powder handling methods.

Further, some results from nanosize HA powder, CAPTAL® R Sintering grade HA (Batch P201), CAPTAL® S Sintering grade HA (Batch P221S BM168) and (β-TCP (Batch P228S) will be presented and discussed in more detail to give an outline of the effects of process parameters.

Figure 2.19 shows scanning electron microscope (SEM) images of different nanobiomaterial powders. The CAPTAL® S has a larger particle size (500 nm–3 μm) due to the sintering and grinding process used during powder manufacture. Merck HA powder and β-TCP have a near-spherical
shape. The CAPTAL® S has an angular shape whereas CAPTAL® R has a long needle shape.

Measurement of angle of repose indicated that all processed powders are cohesive. In particular, the needle shape of CAPTAL® R particles hinders their flow.

All of the nanobiomaterial powder was extruded out as discrete rods due to the strong agglomerations (Fig. 2.20(a)). In the dispensing process, some powder cracks might be formed randomly in the nozzle, as shown in Fig. 2.20(a). The movement difficulties of sticky nanopowder lead to uneven packing densities in different positions of the nozzle (Fig. 2.20(b)). The unpredictable crack would distinctly bring deviations to the amount of dispensed powders for each dose. To reduce the chance of crack forming, the preferential higher amplitude is used.

Dosage can be controlled by adjusting some process parameters but the most efficient way is to change the time of vibration (\(T_V\)). The dosage can be varied simply from a few micrograms to several grams by changing the time of vibration (\(T_V\)) from 0.01 s to a few seconds. Figure 2.21 shows dependency of dose mass on vibration time for the processed nanobiomaterials.

Nozzle size has a significant effect on dispensing of nanobiomaterial powders as well. To reach uniform powder dispensing, nozzle size should be selected appropriately according to particle size/shape and flowability of the nanobiopowder used. Generally, if the nozzle size is too small the powder is dispensed sporadically, and if the nozzle is too big the powder may be over-run. Dose mass change was studied in different nanobiopowders when nozzle size is changed from 1 to 0.8 mm while other process parameters were kept constant. The results show that CAPTAL® S has the highest mean dose mass (and flow rate) at the same flow condition (nozzle size of 1 mm); as seen from the SEM images (Fig. 2.19) it has the biggest particle size, rounded particle shape and less agglomeration which give better flowability. On the other hand, Merck HA nanopowder has the lowest mean dose mass at the same

<table>
<thead>
<tr>
<th>Powder</th>
<th>Particle size</th>
<th>Particle density (kg/m³)</th>
<th>Repose angle</th>
<th>Manufacturing company</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPTAL® S</td>
<td>500 nm–3 μm</td>
<td>–</td>
<td>51.5</td>
<td>Merck KGaA, Germany</td>
</tr>
<tr>
<td>CAPTAL® R</td>
<td>20 nm width-200 nm Length</td>
<td>–</td>
<td>60.3</td>
<td>Merck KGaA, Germany</td>
</tr>
<tr>
<td>Merck HA</td>
<td>50–300 nm</td>
<td>–</td>
<td>55.2</td>
<td>Merck KGaA, Germany</td>
</tr>
<tr>
<td>β-TCP</td>
<td>100–500 nm</td>
<td>–</td>
<td>58.2</td>
<td>Plasma Biotal Limited, UK</td>
</tr>
<tr>
<td>TiO₂</td>
<td>180 nm</td>
<td>4150</td>
<td>38</td>
<td>Tioxide UK Ltd.</td>
</tr>
<tr>
<td>MgO</td>
<td>100 nm</td>
<td>3580</td>
<td>53</td>
<td>PI-KEM Ltd, UK</td>
</tr>
</tbody>
</table>

Source: Li and Yang, 2012.
flow condition (nozzle size of 0.8 mm) as it has the smallest particle size, which forms very strong agglomeration and has the lowest flowability.

### 2.7 Conclusion

In this chapter different SFF techniques suitable for nanobiomaterial processing were reviewed comprehensively. Nanofillers are utilized in SFF
techniques such as SLS, SL, inkjet printing, etc., to control characteristics such as bioactivity, electrical, optical and mechanical properties of 3D printed parts for medical applications. Tissue engineering scaffold fabrication procedures, including material, micro-macrostructure design and manufacturing, were described briefly. Moreover, various extrusion-based SFF processes were classified and their applications in scaffolding investigated extensively. Direct-write assembly and 3D bioplotting are two approaches with great potential as they can process a wide range of biomaterials. Dry powder printing opens up the possibility of producing high resolution, multi-material parts and is a more versatile and realizable alternative manufacturing process for composite scaffold materials.

2.8 References


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