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# Biomanufacturing for tissue engineering: Present and future trends

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Tissue engineering, often referred to as regenerative medicine and reparative medicine, is an interdisciplinary field that necessitates the combined effort of cell biologists, engineers, material scientists, mathematicians, geneticists, and clinicians toward the development of biological substitutes that restore, maintain, or improve tissue function. It has emerged as a rapidly expanding approach to address the organ shortage problem and comprises tissue regeneration and organ substitution. Cells placed on/or within constructs is the most common strategy in tissue engineering. Successful cell seeding depends on fast attachment of cell to scaffolds, high cell survival and uniform cell distribution. The seeding time is strongly dependent on the scaffold material and architecture. Scaffolds provide an initial biochemical substrate for the novel tissue until cells can produce their own extra-cellular matrix (ECM). Thus scaffolds not only define the 3D space for the formation of new tissues, but also serve to provide tissues with appropriate functions. These scaffolds are often critical, both *in vivo* (within the body) or *in vitro* (outside the body) mimicking *in vivo* conditions. Additive fabrication processes represent a new group of non-conventional fabrication techniques recently introduced in the biomedical engineering field. In tissue engineering, additive fabrication processes have been used to produce scaffolds with customised external shape and predefined internal morphology, allowing good control of pore size and pore distribution. This article provides a comprehensive state-of-the-art review of the application of biomanufacturing additive processes in the field of tissue engineering. New and moving trends in biomanufacturing technologies and the concept of direct cell-printing technologies are also discussed.

*Keywords:* Biomanufacturing; biomaterials; scaffolds; tissue engineering

## 1. Introduction

Tissue engineering is a multidisciplinary field that requires the combined effort of cell biologists, engineers, material scientists, mathematicians, geneticists, and clinicians toward the development of biological substitutes that restore, maintain, or improve tissue function. Initially defined by Skalak and Fox (1988) as 'the application of principles and methods of engineering and life sciences toward the fundamental understanding of structure–function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve tissue function', it is a major component of regenerative medicine. Diseases such as Parkinsons, Alzheimers, osteoporosis, spine injuries or cancer, might in the near future be treated with methods that aim at regenerating diseased or damaged tissues.

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Tissue engineering comprises three main strategies (Mistry and Mikos 2005, Matsumoto and Mooney 2006, Bártolo *et al.* 2007):

- *Cell-based strategies*, which involves the direct *in vivo* implantation of isolated cells or cell substitutes and it is based on cells synthesizing their own matrix. Tissues are typically built from several types of cells with tissue specific composition and alignment in both two and three dimensions, and contain a similarly tissue-specific extracellular matrix (ECM).
- *Growth-factor-based strategies*, which are based on growth factors and controlled-released systems. Growth factors are signalling molecules that regulate a multitude of cellular functions such as proliferation, differentiation, migration, adhesion and gene expression.
- *Scaffold-based strategies*, which is based on the use of a temporary scaffold that provides a substrate for the implanted cells and a physical support to organize the formation of the new tissue. In this approach, transplanted cells adhere to the scaffold, proliferate, secrete their own ECM matrices and stimulate new tissue formation.

Scaffolds are often critical, both *ex vivo* as well as *in vivo*, as they serve some of the following purposes (Leong *et al.* 2003, Bártolo *et al.* 2007, 2009): allow cell attachment, proliferation and differentiation (Figure 1); deliver and retain cells and growth factors; enable diffusion of cell nutrients and oxygen and enable an appropriate mechanical and biological environment for tissue regeneration in an organised way. To achieve these goals an ideal scaffold must satisfy some biological and mechanical requirements as shown in Table 1. In order to meet the requirements stated for biomimicry, strategies are developed to optimize control in scaffold architecture designs, in terms of macro- and microstructure (Yang *et al.* 2001, Yeong *et al.* 2004).

Scaffold-based strategies for tissue engineering strongly depend on both materials and manufacturing processes. Scaffold materials have included polymers and ceramic materials. Some of the most popular polymers are the aliphatic polyesters such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(caprolactone) (PCL), poly(lactide-co-glycolide) (PLGA), polysaccharides such as starch, alginate, hyaluronic acid and chitosan, and proteins such as collagen and gelatine (Nair and Laurencin 2006, Velema and Kaplan 2006, Chan and Mooney 2008). The two most commonly used ceramics are hydroxyapatite (HA) and β-tricalcium phosphate (TCP) (Figure 2). However bioceramic materials are usually brittle and difficult to process into porous structures with complex shapes. Scaffolds made of composites with different polymers, as well as polymer/ceramics blends have also been fabricated to widen the range of mechanical properties achievable. Among the hybrids attempted are PCL/HA (Wiria *et al.* 2007), polyetheretherketone/hydroxyapatite (PEEK/HA) (Tan *et al* 2003),

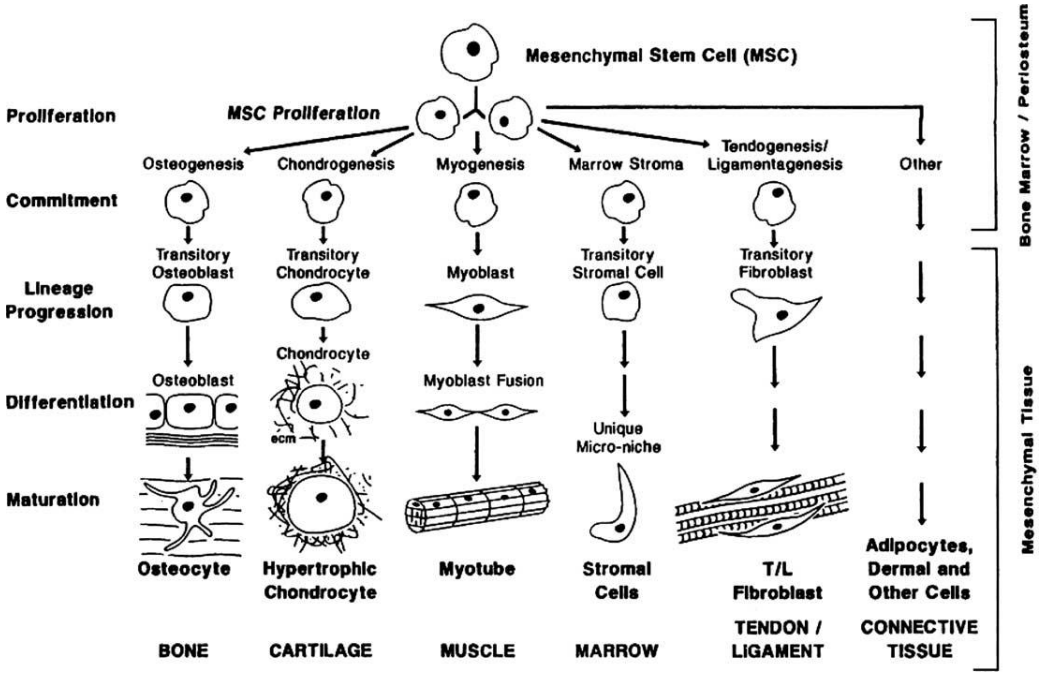


Figure 1. Mesenchymal stem cells differentiation process in response to cues from the cellular environment (Nikovits and Stockdale 2007).

Table 1. Biological, mechanical and physical requirements (Mistry and Mikos 2005, Bártolo *et al.* 2007, 2009).

| | |
|---|---|
| Biological requirements | |
| *Biocompatibility* | The scaffold material must be non-toxic and allow cell attachment, proliferation and differentiation |
| *Biodegradability* | The scaffold material must degrade into non-toxic products |
| *Controlled degradation rate* | The degradation rate of the scaffold must be adjustable in order to match the rate of tissue regeneration. Controlled degradation of a scaffold allows for gradual |
| *Porosity* | Appropriate porosity macro and microstructure of the pores and shape to allow tissue in-growth and vascularisation. Scaffolds must be designed to maximise porosity while maintaining mechanical properties |
| Mechanical and physical requirements | |
| *Strength and stiffness* | Sufficient strength and stiffness to withstand stresses in the host tissue environment. Mechanical properties of a scaffold must initially match the properties of the target tissue to provide structural stability to an injury site |
| *Surface Finish* | Adequate surface finish to guarantee that a good biomechanical coupling is achieved between the scaffold and the tissue |
| *Sterilised* | Easily sterilised either by exposure to high temperatures or by immersing in a sterilisation agent remaining unaffected by either of these processes. The sterilisation process must not alter the material's chemical composition, as this may affect its bioactivity, biocompatibility or degradation properties |

poly(L-lactide-co-D,L-lactide)/tricalcium phosphate (PLDLLA/TCP) (Lam *et al.* 2008), PCL/PEO (Rath *et al.* 2008), and chitosan/gelatin (He *et al.* 2008). The scaffold design and fabrication methodologies involved will have to take into consideration the range of materials viable, in addition to the specific structure and function of the tissue of interest (Yang *et al.* 2002, Yeong *et al.* 2004).

In this paper, we review and compare various advanced fabrication techniques to produce 3D scaffolds for tissue engineering. Future trends and directions are also discussed following the comparison in the progress of these advances in tissue engineering.

## 2. Conventional Techniques To Produce Scaffolds

Conventional methods to fabricate scaffolds include (Bártolo *et al.* 2007, 2009, Engel *et al.* 2007):

- Solvent casting/salt leaching: involves mixing solid impurities, such as sieved sodium chloride particles, into a polymer solvent solution, and casting the dispersion to produce a membrane of polymer and salt particles. The salt particles are then leached out with water to yield a porous membrane. Porosity and pore size have been shown to be dependent on salt weight fraction and particle size.
- Phase separation: involves dissolving a polymer in a suitable solvent, placing it in a mould, and then cooling the mould rapidly until the solvent is frozen. The solvent is removed by freeze-drying, leaving behind the polymer as foam with pore sizes of 1–20 μm in diameter.
- Foaming: is carried out by dissolving a gas, usually $CO_2$, at elevated pressure or by incorporating a chemical blowing agent that yields gaseous decomposition products. This process generally leads to pore structures that are not fully interconnected and produces a skin-core structure.
- Textile meshes: these processes include all technologies successfully employed to fabricate non-woven meshes of different polymers. Major limitations are due to difficulties in obtaining high porosity and regular pore size.

Each of these techniques presents several limitations as they usually do not enable to properly control pore size, pore geometry and spatial distribution of pores, besides being almost unable to construct internal channels within the scaffold. Although the shape and the size of the pores can be varied by changing the parameters of these techniques, the resulting scaffold organisation of pores is random, leading to inconsistencies in scaffold architecture. This can lead to pore pathways that are only partially connected and that follow contorted routes, which could impede the supply

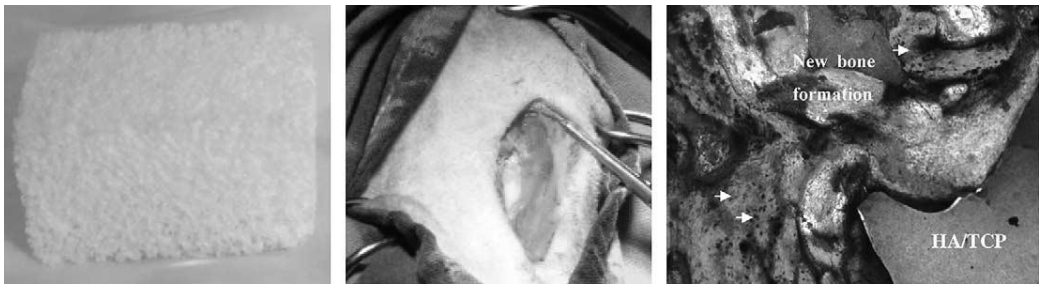


Figure 2. An interdisciplinary group of researchers from the Polytechnic Institute of Leiria, University of Aveiro and University of Évora, Portugal, have been evaluated the behaviour of bioceramic scaffolds (HA, TCP and HA-TCP) for bone regeneration.

of nutrients and the ingrowth of tissue into the scaffold. Beyond these limitations, these techniques usually involve the use of toxic organic solvents, long fabrication times on top of being labour-intensive processes. Therefore, biomanufacturing additive fabrication processes are considered as viable alternatives to fabricate scaffolds for tissue engineering as they offer better control and the ability to actively design the porosity and interconnectivity of scaffolds. Aside from being design dependent as opposed to the process dependent tendency of conventional techniques, biomanufacturing additive processes allow a good range of processible materials as well as repeatable defined microarchitectures (Yang *et al.* 2002).

## 3. Biomanufacturing additive processes

Biomanufacturing additive processes represent a new group of non-conventional fabrication techniques recently introduced in the biomedical engineering field. The main advantages of these techniques are both the capacity to rapidly produce very complex 3D models in a layer-by-layer fashion and the ability to use various raw materials. When combined with clinical imaging data, these fabrication techniques can be used to produce constructs that are customised to the shape of the defect or injury. Some processes operate at room temperature, thus allowing for cell encapsulation and biomolecule incorporation without significantly affecting viability. In the tissue engineering field, biomanufacturing additive processes have been used to produce scaffolds with customised external shape and predefined internal morphology, allowing good control of pore size and pore distribution (Bártolo 2006). These techniques include stereolithographic processes, laser sintering, extrusion and three dimensional printing.

### 3.1 *Stereolithographic processes*

Stereolithographic processes produce three-dimensional solid objects in a multi-layer procedure through the selective photo-initiated cure reaction of a polymer (Bártolo and Mitchell 2003). These processes usually employ three distinct methods of irradiation. The first method is the mask-based method in which an image is transferred to a liquid polymer by irradiating through a patterned mask. The irradiated part of the liquid polymer is then solidified. In the second method, a direct writing process using a focused UV beam produces polymer structures. The third method utilises inkjet technology in which photopolymer materials are jetted onto a building tray forming the designed architecture.

The direct or laser writing approach consists of a vat containing a photosensitive polymer, a moveable platform on which the model is built, a laser to irradiate and cure the polymer and a dynamic mirror system to direct the laser beam over the polymer surface 'writing' each layer. After drawing a layer, the platform dips into the polymer vat, leaving a thin film from which the next layer will be formed. Mask-based writing systems build models by shining a flood lamp through a mask, which lets light pass through it. These systems generally require the generation of a lot of masks with precise mask alignments. One solution for this problem is the use of a liquid crystal display (LCD) or a digital processing projection system as a flexible mask (Figure 3). For Objet's PolyJet inkjet technology, each photopolymer layer is cured by UV irradiation immediately after jetting, forming fully cured structures that require no post curing after completion of the build. The gel-like support material, which is required for complicated geometrical structures, can be easily separated from the main build material by hand and water jetting (Figure 4) (Tan *et al.* 2009).

Levy *et al.* (1997) used a direct irradiation stereolithographic process to produce HA ceramic scaffolds for orbital floor prosthesis. A suspension of fine HA powder into a UV-photocurable resin was formulated and used as building material. The photo-cured resin acts as a binder to hold the HA particles together. The resin is then burnt out and the HA powder assembly sintered for consolidation. A similar approach was used by Griffith and Halloran (1996) that produced ceramic scaffolds using suspensions of alumina, silicon nitride and silica particles with a photo-curable resin. The binder was removed by pyrolysis and the ceramic structures sintered.

Stereolithography is commonly used to produce a negative replica that is filled typically with ceramic slurries and burnt away during sintering. Chu *et al.* (2001) developed a lost-mould technique to produce implants with designed channels and connection pattern. Stereolithography was used to create epoxy moulds designed from negative image of implants. A highly loaded HA-acrylate suspension was cast into the mould. The mould and the acrylic binder were removed by pyrolysis and the HA green scaffold submitted to a sintering process. The finest channel size achieved was about 366 μm and the range of implant porosity between 26 and 52%.

More recently, indirect fabrication of natural polymeric scaffolds for soft tissues has been studied. Tan *et al.* (2009) developed an approach utilising Objet's inkjet technology coupled with a foaming process to fabricate gelatin scaffolds. Through the reverse lost mould technique, a first mould was fabricated via rapid prototyping to serve as a negative for a second paraffin mould. Gelatin prepared by a controlled foaming process was then casted into the second mould to form scaffolds with 3D interconnected channels. The application of a foaming material combined with Objet's droplet based technology produced uniformly porous scaffolds with complex channel architectures. Cytotoxicity test

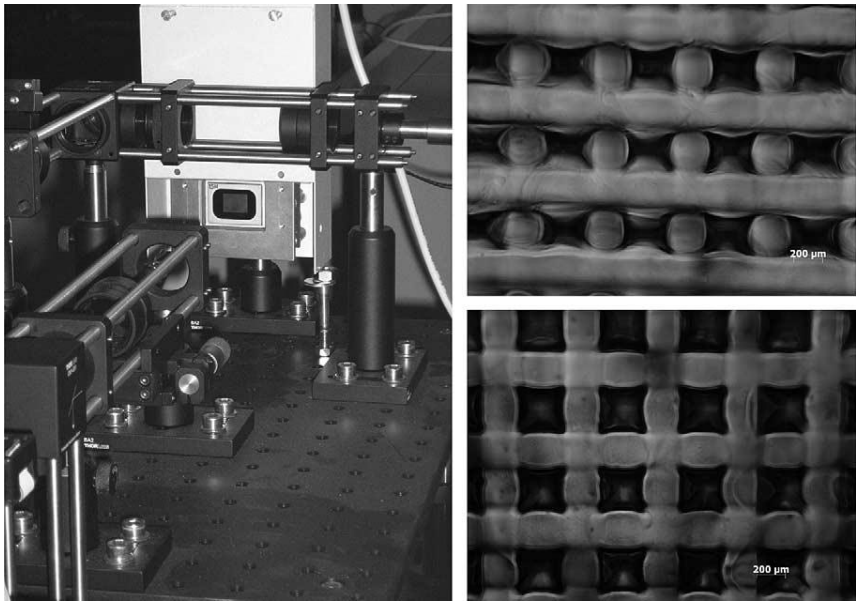


Figure 3. Mask-based writing system and polyHEMA constructs produced at the Polytechnic Institute of Leiria.

results showed that multiple processes involved in the scaffold fabrication had not induced cell toxicity.

The direct fabrication of biopolymeric scaffolds has also been reported. In another study, Cooke *et al.* (2002) used a biodegradable resin mixture of diethyl fumarate, poly(propylene fumarate) and bisacylphosphine oxide as photoinitiator to produce scaffolds for bone ingrowth. Lan *et al.* (2009) produced poly(propylene fumarate) (PPF) scaffolds with highly interconnected porous structure and porosity of 65%. The scaffolds were coated by applying accelerated biomimetic apatite and arginine–glycine–aspartic acid peptide coating to promote cell behaviour. The coated scaffolds were seeded with MC3T3-E1 pre-osteoblasts and their biological properties were evaluated using an MTS assay and histological staining. Melchels *et al.* (2009) used a resin based on poly(D,L-lactide) macromonomers and non-reactive diluent to produce porous scaffolds with gyroid architecture (Figure 5). It was also possible to observe that pre-osteoblasts readily adhered and proliferated well on these scaffolds.

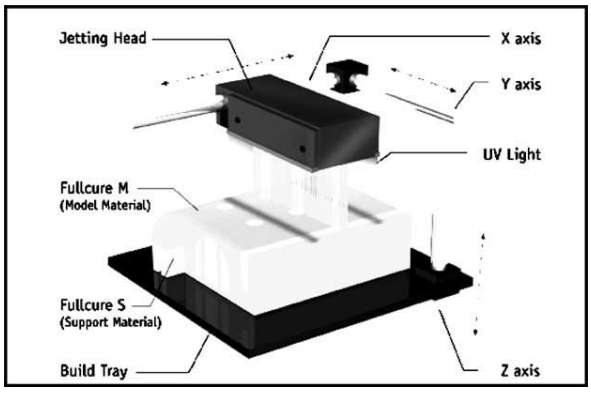


Figure 4. The Objet PolyJet inkjet technology-based process.

The photopolymerisation of biomaterials via multiphoton excitation also provide an efficient method of scaffold microfabrication. In this process the beam of an ultra-fast infrared laser is tightly focused into the volume of a photosensitive material (Figure 6). The polymerisation process can be initiated by non-linear absorption within the focal volume. Multiphoton excitation has been applied to fabricate scaffolds of a wide range of polymers and bulk protein formulations, such as collagen, laminin, fibronectin, bovine serum albumin, alkaline phosphatase, etc.

Mask-based writing system can be used to pattern hydrogel structures with high resolution. Liu and Bhatia (2002) reported a method, where multiple steps of micropatterned photopolymerisation processes can be coupled to produce 3D cell matrix structures with micro-scale resolution (Figure 7).

### 3.2 *Laser sintering*

Selective laser sintering (SLS) uses a laser emitting infrared radiation, to selectively heat powder material just beyond its melting point. The laser traces the shape of each cross-section of the model to be built, sintering powder in a thin layer. It also supplies energy that not only fuses neighbouring powder particles, but also bonds each new layer to those previously sintered. After each layer is solidified, the piston over the model retracts to a new position and a new layer of powder is supplied using a mechanical roller. The powder

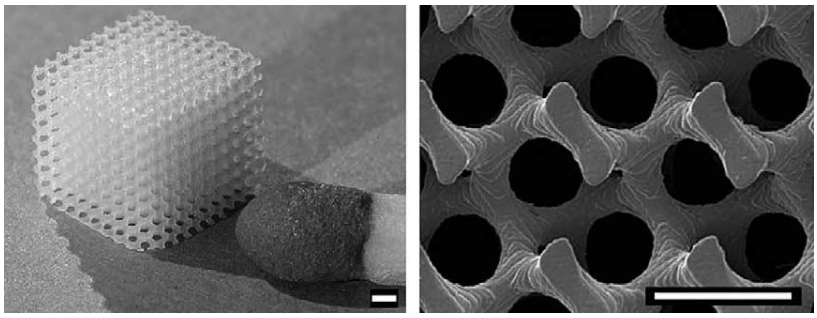

Figure 5. PDLLA scaffolds with a gyroid architecture built by stereolitography. Scale bars represent 500 μm (Melchels *et al.* 2009).

that remains unaffected by the laser acts as a natural support for the model and remains in place until the model is complete.

The potential of SLS to produce PCL scaffolds for replacement of skeletal tissues was shown by Williams *et al*. (2005). The scaffolds were seeded with bone morphogenetic protein-7 (BMP-7) transduced fibroblasts. *In vivo* results showed that these scaffolds enhance tissue ingrowth, on top of possessing mechanical properties within the lower range of trabecular bone. Compressive modulus (52–67 MPa) and yield strength (2.0–3.2 MPa) were in the lower range of properties reported for human trabecular bone.

In addition, the feasibility of fabricating scaffolds with PCL/HA biocomposites on SLS has been investigated by Wiria *et al.* (2007). HA particles were added to increase the bioactivity of the overall scaffold. These powders were mechanically blended at varying ratios (10, 20 and 30 wt.% HA) and processed under varying machine parameters. Optimization and cell proliferation studies with Saos-2 cells were carried out on sintered and sterilized PCL/HA with 10 wt.% HA. Studies were also carried out on PVA/HA biocomposites for bone replacement applications (Chua *et al.* 2004, Wiria *et al.* 2008). PVA powder was grinded to varying particle sizes prior to mixing with 10 wt.% HA. Fourier transform infrared spectrometer (FTIR) and scanning electron microscopy (SEM) results indicated that neither the physical grinding nor laser sintering compromised the chemical composition of PVA.

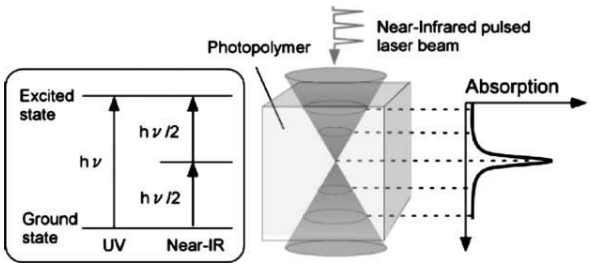


Figure 6. Principle of multiphoton polymerisation.

Powder blends of PEEK/HA have also been processed experimentally on SLS (Figure 8) (Tan *et al.* 2003, 2005, Naing *et al.* 2008). Mixtures of the powders were obtained through mechanical blending using a roller-mixer. High porosity and pore interconnectivity obtained for the specimens were attributed to use of powder stocks and relatively low compaction forces exerted on powder bed during the additive processes. The studies were carried out to determine the potential of sintering a high melting point biopolymer in lower temperature environment.

Lee and Barlow (1996) coated calcium phosphate powder with polymer by spray drying slurry of particulate and emulsion binder. The coated powder was then sintered to fabricate calcium phosphate bone implants. Afterwards, these structures were infiltrated with calcium phosphate solution or phosphoric acid-based inorganic cement.

Zhou *et al.* (2008) studied the use of bio-nano-composite microspheres, consisting of carbonated hydroxyapatite (CHAp) nanospheres within a PLLA matrix to produce scaffolds (Figure 9). PLLA microspheres and PLLA/CHAp nanocomposites microspheres were prepared by emulsion techniques. The resultant microspheres had a size of 5–30 μm, suitable for the SLS process. The use of PLLA/CHAp nanocomposite microspheres seems to offer a solution to the problem of removing the excessive powder from the pores after fabrication.

### 3.3 *Extrusion-based processes*

The extrusion-based technique, commercially known as fused deposition modelling (FDM), was developed by Crump (1992). By this process, thin thermoplastic filaments are melted by heating and guided by a robotic device controlled by a computer, to form the object. The material leaves the extruder in a liquid form and hardens immediately. The previously formed layer, which is the substrate for the next layer, must be maintained at a temperature just below the solidification point of the thermoplastic material to assure good interlayer adhesion.

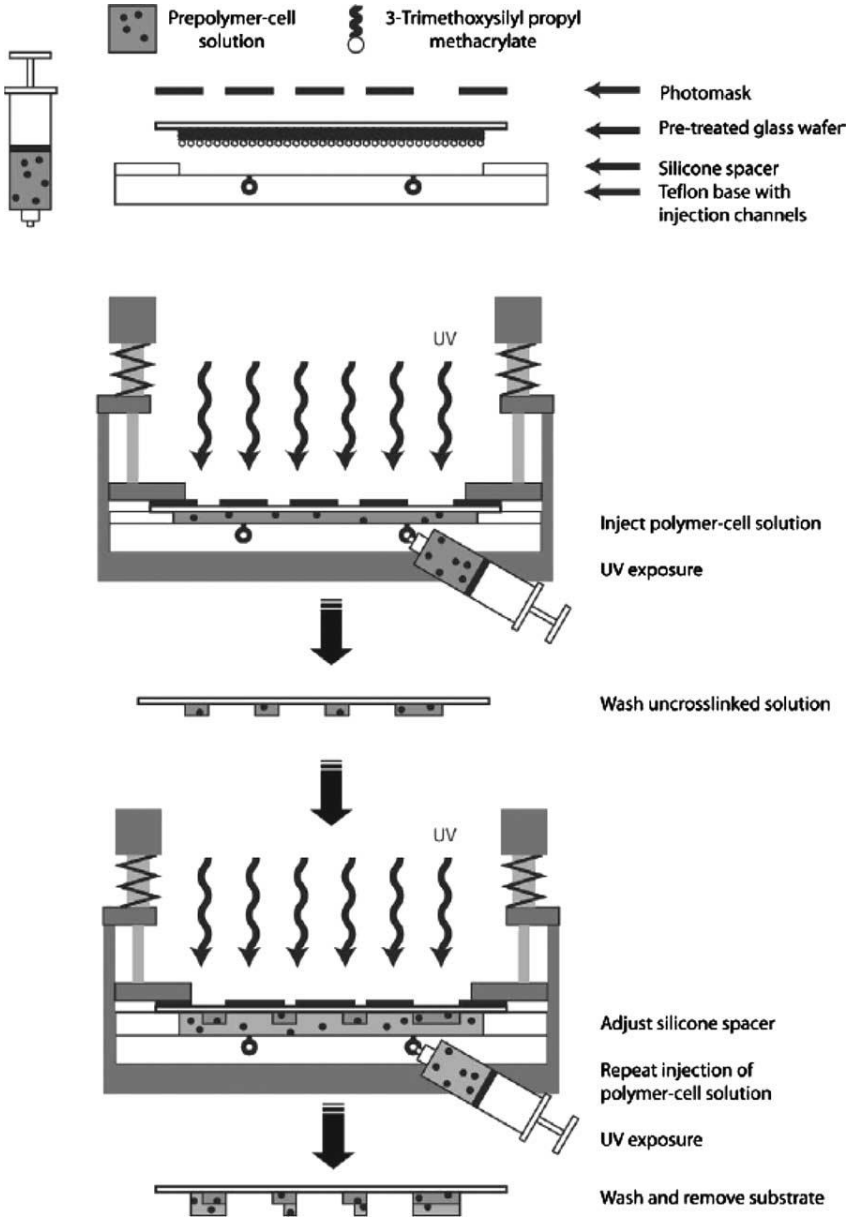


Figure 7. Process for formation of hydrogel microstructures containing living cells (Liu and Bhatia 2002).

Ramanath *et al.* (2007, 2008) have previously studied the melt flow behaviour of polymers for biomedical applications. By comparing results from mathematical modelling and finite element analyses performed based on scaffold material properties of PCL and FDM machine parameters, they found that the pressure gradient along the melt flow channel was directly influenced by the force feeding the filaments, depositing nozzle diameter and angle variation. Their studies concluded that the quality and consistency of scaffold built would be affected without a pressure drop feedback system integrated in the FDM extrusion head. Although being capable of fabricating various honeycomb-like architectures, the pore morphologies for scaffolds created by FDM were not consistent along the 3 dimensions when applying the standard system software build patterns. One method to overcome this is to stack consecutive material layers of similar lay down pattern and change the pattern subsequently when the desired pore morphology is produced (Chua *et al.* 2009).

Extrusion-based processes have been used to successfully produce scaffolds in PCL, PP-TCP, PCL-HA, PCL-TCP with resolution of 250 μm. Some of the major limitations

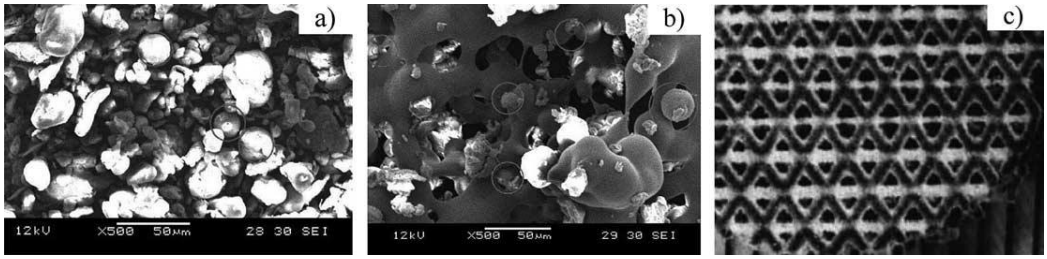


Figure 8. SEM micrographs of PEEK-10% wt HA (a) before and (b) after sintering. (c) Bottom view of PEEK-HA composite scaffold (Naing *et al.* 2008, Tan *et al.* 2003).

of FDM are due to the use of filament-based materials and the high heat effect on raw material. In order to solve some limitations of the FDM process, such as the requirement of precursor filaments or high processing temperatures, some alternative processes have been proposed.

Woodfield *et al.* (2004) used a FDM-like technique, called 3D fibre deposition, to produce poly(ethylene glycol)-terephthalate-poly(butylenes terephthalate) (PEGT/PBT) block co-polymer scaffolds with a 100% interconnecting pore network for engineering of articular cartilage. By varying the co-polymer composition, porosity and pore geometry, scaffolds were produced with a range of mechanical properties close to articular cartilage. The scaffolds seeded with bovine chondroccytes supported a homogeneous cell distribution and subsequent cartilage-like tissue formation.

Drexel University developed a variation of FDM called precision extruding deposition for fabrication of bone tissue scaffolds. In this process, material in pellet or granule form is fed into a chamber where it is liquefied. Pressure from a rotating screw forces the material down a chamber and out through a nozzle tip. This process was used by Wang *et al.* (2004) to directly fabricate PCL scaffolds with controlled pore size of 250 µm and designed structural orientations (0°/90°, 0°/120° or both combined patterns). Proliferation studies were performed using cardiomyoblasts, fibroblasts and smooth muscle cells. PED was also used by Yildirim *et al*. (2008) to fabricate PCL scaffolds coupled with surface plasma treatment in order to enhance osteoblast adhesion and proliferation.

Tellis *et al* (2008) used micro CT to create biomimetic tissue engineering scaffolds. CAD models were exported to a FDM machine, producing polybutylene terephthalate (PBT) trabecular scaffolds. The scaffolds were compression tested at two different load rates (49 and 294 N/s). Some scaffolds were soaked in a 25°C saline solution for 7 days before compression. When compressed at 49 N/s the dry trabecular scaffolds had a compressive stiffness ranging from $2.46 \pm 0.55$ MPa for the complex interconnected pore structure (case E in Figure 10) to $5.11 \pm 1.89$ MPa for the simple linear structure (case A in Figure 10). At 294 N/S, the compressive stiffness values roughly doubled. It was also observed that soaking the scaffolds in saline solution had an insignificant effect on stiffness and that compressive stiffness decreased as pore size increased. Compressive trabecular scaffolds matched bone samples in porosity. However, physiologic connectivity density and trabecular separation requires optimisation of scaffold processing.

The Polytechnic Institute of Leiria developed a variation of FDM called BioExtruder (Mota *et al.* 2009). It is a highly

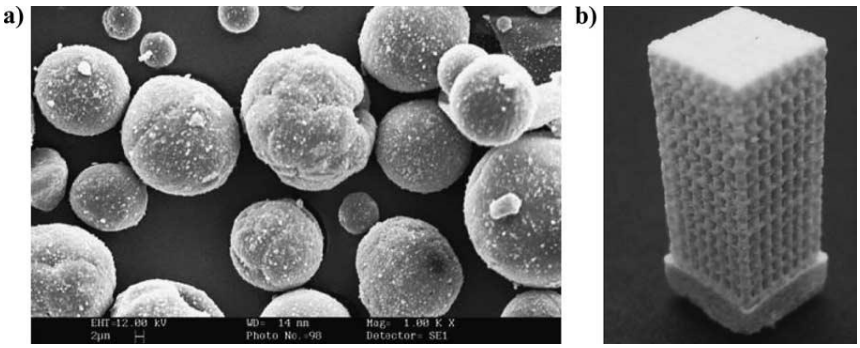


Figure 9. (a) SEM image of PLLA/CHAp nanocomposite microspheres. (b) PLLA/CHAp nanocomposite scaffolds (Zhou *et al.* 2008).

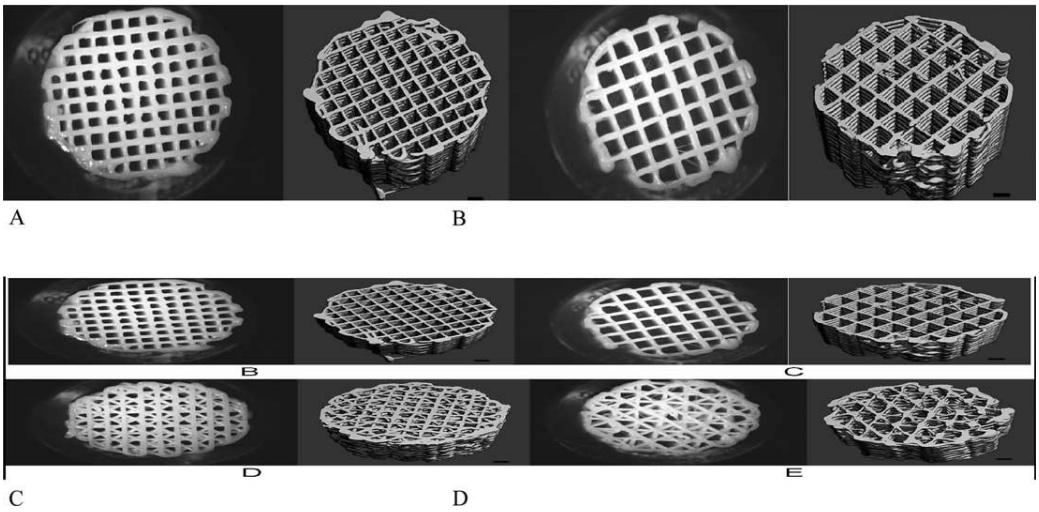


Figure 10. Digital photographs and micro CT 3D segmentations of PBT scaffolds (Tellis *et al.* 2008).

reproducible and low cost system enabling the controlled definition of pores into the scaffold to modulate mechanical strength and molecular diffusion, as well the fabrication of multi-material scaffolds. It 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 (Figure 11a and b). Fibroblasts were successfully attached to the scaffolds (Domingos *et al.* 2009). The BioExtruder system is also being used in combination with an electrospinning process to produce well behaved scaffolds (Figure 11c) (Mota *et al.* 2009).

Ang *et al.* ( 2002) developed a rapid prototyping robotic dispersing system using the same principle as the 3D bioplotting system, which was used to produce chitosan-HA scaffolds. Solutions of chitosan-HA were extruded into a sodium hydroxide and ethanol medium to induce the precipitation of chitosan. The scaffolds were then hydrated, frozen and freeze-dried.

An alternative process has been developed by Mironov *et al.* (2003) and Yan *et al.* (2003), which developed the concept of cell printing. This process prints gels, single cells and cell aggregates offering a possible solution for organ printing. An analogous process, called alginate-based rapid prototyping, has been developed at the Polytechnic Institute of Leiria. This process produces alginate solid structures, by extruding a previously prepared solution of sodium alginate in water, mixed with a solution of calcium chloride, providing a temporary support for the seeded cells in culture (Rezende *et al.* 2009).

### 3.4 *Three-dimensional printing*

Three-dimensional printing (3DP) was developed at the Massachusetts Institute of Technology (USA) by Sachs *et al.* (1993). The process deposits a stream of microparticles of a binder material over the surface of a powder bed, joining particles together where the object is to be formed. A piston lowers the powder bed so that a new layer of

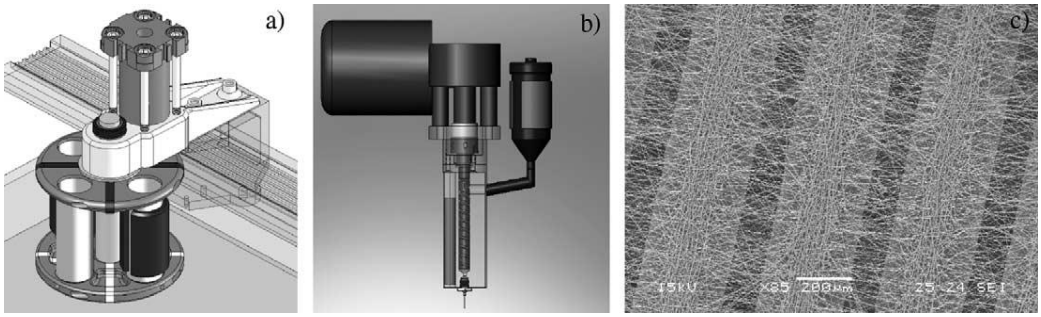


Figure 11. (a) Multi-material extrusion system; (b) Single-material extrusion; (c) SEM of PLGA nanofibers (deposition time 40 s, feed rate = 4 ml/h) above a $30 \times 30 \times 4$ mm PCL scaffold (pore size 150 μm).

powder can be spread over the surface of the previous layer and then selectively joined to it.

Kim *et al.* (1998) employed 3DP with particulate leaching to create porous scaffolds, using polylactide-coglycolide (PLGA) powder mixed with salt particles and a suitable organic solvent. The salt particles were leached using distilled water. Cylindrical scaffolds measuring 8 mm (diameter) by 7 mm (height) with pore sizes of 45–150 μm and 60% porosity were fabricated. Hepatocytes were successfully attached to the scaffolds.

The influence of pore size and porosity on cell adhesion and proliferation were investigated by Zeltinger *et al.* (2001). Disc shaped poly(L-lactic acid) (L-PLA) scaffolds measuring 10 mm (diameter) by 2 mm (height) were produced through both 3DP and salt and leaching methods. The scaffolds were produced with two different porosities (75 and 90%) and four different pore size distributions (<38, 38–63, 63–106 and 106–150 μm), and tested with cell culture using canine dermal fibroblasts, vascular smooth muscle cells and microvascular epithelial cells.

Lam *et al.* (2002) developed a blend of starch-based powder containing cornstarch (50%), dextran (30%) and gelatine (20%), bounded by printing distilled water. Cylindrical scaffolds were produced measuring 12.5 mm (diameter) by 12.5 mm (height) and infiltrated with different amounts of a copolymer solution consisting of 75% L-PLA and 25% polycaprolactone in dichloromethane to improve their mechanical properties.

Sachlos *et al.* (2003) used an indirect approach to produce collagen scaffolds with complex internal morphology and macroscopic shape by using a 3DP sacrificial mould. A dispersion of collagen was cast into the mould and frozen. The mould was then dissolved with ethanol and the collagen scaffold was critical point dried with liquid $CO_2$.

Another processing system based on inkjet printing technology is the 3D phase change inkjet printer developed by Solidscape, Inc (USA). This process utilises droplet deposition technique in which thermoplastic building material and a wax like support material are deposited from separate jets onto a working surface. As a result of heat conduction, the droplets induces local melting on the underlying layer and causes bonding to occur. After each layer hardens, uniform thickness is maintained by a milling head. ModelMakerII$^{TM}$ (MMII) is a representative system (Yeong *et al.* 2004).

Yeong *et al.* (2006, 2007) also utilised a similar indirect approach with MMII to fabricate collagen scaffolds by creating a sacrificial mould designed with interconnected channels incorporated throughout the scaffold. In addition, they investigated different drying routes after removal of the sacrificial mould with ethanol. The effect of freeze drying process after immersion of the scaffolds in distilled water, and critical point drying with $CO_2$ was observed through dimensional shrinkage, pore size distribution and morphology analysis.

## 4. New and moving trends

Additive biomanufacturing has the added advantage of automation in the design and fabrication of patient specific scaffolds. Besides having the ability to rapidly fabricate complex exterior geometries for scaffold implants, more importantly, it enables the manufacturing of complex porous microstructures. These complex interior microarchitectures required of the scaffolds depends on the mechanical properties, physical and molecular cues of the surrounding tissue at the defect site. Thus, one way to achieve such hierarchical designs is to create libraries of unit cells that can be assembled with an algorithm program, and henceforth interface it with the various biomanufacturing modalities (Cheah *et al.* 2004, Hollister 2005, Adachi *et al.* 2006, Bucklen *et al.* 2008). Naing *et al.* (2008) developed a novel computer-aided design (CAD) library system of such structures based on open polyhedral units, which they named as computer aided system for tissue scaffolds (CASTS).

Taking into consideration the natural non-homogeneous nature of tissues and organs not only anatomically, but in terms of biological and mechanical characteristics as well, Leong *et al.* (2008) addressed the need and necessity to fabricate scaffolds with functional gradients. Scaffolds integrating such architectural cues are pertinent to facilitate the dynamics of tissue development in vitro and allow the formation of more complex tissues. Chua *et al.* (2009) developed a model for designing functionally graded tissue scaffolds using CASTS as the designing platform. They compiled data of compressive stiffness and strength versus porosity relations of structures assembled from the polyhedral unit cells. These were then used as a database to create scaffolds of corresponding stiffness gradient to that of the bone defects obtained from medical imaging. The regeneration of human mandibular cancellous bone was demonstrated as a case study. In another approach, Cai *et al.* (2008) used a finite element-based method by applying hexahedral mesh refinements on macroporous scaffold morphologies to create a control for pore size distribution in bone scaffolds, hence creating porosity gradients.

The versatility of additive biomanufacturing processes has improved the range and diversity of scaffolds that can be generated. These enhancements enable a better understanding of the design parameters such as scaffold integrity, surface properties and pore architectures needed to engineer tissues that mimic the native body's healing and development. Low temperature operations have also been explored to preserve temperature sensitive and bioactive components in tissue engineering (Lim *et al.* 2008). Cell-based additive

biomanufacturing have been intensively studied and many innovative process techniques have surfaced to complement limitations in purely scaffold based approaches. Among the new potential technologies are organ bioprinting, laser writing of cells, bio-electrospraying, hydrogel micropatterning, and microvasculature fabrication.

### 4.1 *Organ bioprinting*

Based on the inherent self assembling capacity of cells and tissues in mimicking natural morphogenesis, Mironov *et al.* (2007) outlined a process framework involving material, substrate, printer and bioreactor to implement the technology. Tissue aggregates in spheroid forms were advocated as printing material serving as the fundamental building blocks to be printed layer by layer with digital robotic bioprinters on hydrogels that allow tissue fusion (Mironov *et al.* 2009). The authors also discussed the feasibility of incorporating intraorgan vascular networks by engineering vascularised tissue spheroids.

### 4.2 *Laser writing of cells*

Using an ArF excimer laser with the technique termed as matrix-assisted pulsed laser evaporation direct write (MAPLE DW), Schiele *et al.* (2009) were able to write cells onto cellular constructs with micropatterns repeatably and uniformly. Different cell types, including dermal fibroblasts, myoblasts, neural stem cells, and breast cancer cells used in their study demonstrated potential in directed cellular assembly and multi-cell culture for cell-based additive manufacturing.

### 4.3 *Bio-electrospraying*

This new technique developed at the University College London, applies high intensity electric field between a conducting needle which contains the flowing material within, and a ground electrode that can take shape of a ring, which induces jetting of the material causing the formation of a spray (Jayasinghe 2007, Abeyewickremea *et al.* 2009). Cell suspensions can be directly handled with this approach. Investigations with murine embryonic stem cells reported cell viability of 80-88% and no significant effects on pluripotency.

### 4.4 *Hydrogel micropatterning*

Barry *et al.* (2009) designed a photopolymerizable hydrogel solution containing acrylamide that enables further improved direct ink writing. The solution is deposited through a gold-coated micronozzle in accordance to designed scaffold micro features layer by layer and simultaneously irradiated by UV light. The hydrogel filament meshes created were of 1 μm wide with a spacing of 5 μm in between, and arranged to delineate correct tissue structure. They were coated with Poly-D-lysine and cell seeding with murine fibroblast cells showed the cells aligning to the scaffold patterns. The laser direct-write photopolymerisation and multiple-beam laser interference photopolymerisation method was used in another study to fabricate submicron range structures on polyethylene glycol diacrylate (PEG-DA) (Yuan *et al.* 2008). Hydrogel micropatterning has also been carried out with electrowetting-based microfluidics array printing for soft tissue engineering purposes (Zhou *et al.* 2007).

### 4.5 *Microvasculature fabrication*

Among the major concerns in the current tissue engineering paradigm is the incorporation of vasculature in successfully engineered thick tissue constructs to meet mass transport requirements. Cui *et al.* (2009) used a modified thermal inkjet printer and demonstrated the feasibility of printing microvasculature with human microvascular endothelial cell suspensions in thrombin solutions onto fibrinogen solutions which served as the substrate. The printed cells achieved the capacity to interact and proliferate within fibrin channels forming a tubular lining. In order to automate the design of branched vascular trees, Mironov *et al.* (2009) recently investigated the degree of tissue retraction in tubular-like constructs, formed from the fusion of tissue spheroids, to be incorporated into CAD.

## 5. Conclusion

Additive biomanufacturing has been established as a viable set of processes to produce three-dimensional scaffolds of various materials, including polymers, hydrogels, ceramics and even cells. These technologies offer a high degree of freedom for tissue engineering either for the design of scaffolds (pore size, pore geometry, orientation, interconnectivity, etc.) or for its fabrication. Scaffold fabrication using these techniques allows the possibility of indirectly controlling macroscopic properties, such as the bulk mechanical properties and the permeability for nutrient transport considerations. These characteristics can enhance the fabrication of biomimetic scaffolds, functionally graded scaffolds and scaffolds for complex biomechanical applications. The emergence of these technologies in tissue engineering also increases the need for adaptation of the existing standards.

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