3D fiber deposition technique to make multifunctional and tailor-made scaffolds for tissue engineering applications

Antonio Gloria1, Teresa Russo2, Roberto De Santis1, Luigi Ambrosio1

1Institute of Composite and Biomedical Materials, National Research Council, Naples - Italy
2Department of Materials and Production Engineering, University of Naples “Federico II”, Naples - Italy

ABSTRACT: Tissue engineering represents an interesting approach which aims to create tissues and organs de novo. In designing scaffolds for tissue engineering applications, the principal goal is to mimic the function of the natural extracellular matrix, providing a temporary template for the growth of target tissues. For this reason, scaffolds should possess suitable mechanical properties and architecture to play their specific role. In this paper, limitations of conventional scaffold fabrication methods will be briefly introduced, and rapid prototyping techniques will be described as advanced processing methods to realize customized scaffolds with controlled internal microarchitecture. Among the rapid prototyping techniques, the potential and challenges of 3D fiber deposition to create multifunctional and tailor-made scaffolds will be reviewed. (Journal of Applied Biomaterials & Biomechanics 2009; 7: 141-52)

Key words: Tissue engineering, Scaffolds, Rapid prototyping, 3D fiber deposition, Bioplotter

INTRODUCTION

Tissue engineering has been defined as a multidisciplinary field that integrates principles of engineering and life sciences to develop biological substitutes that restore, maintain or improve tissue function (1-3). It aims to overcome the limitations of conventional treatments based on organ transplantation and biomaterial implantation (1, 3), and has the potential to produce a supply of immunologically tolerant “artificial” organs and tissue substitutes that can grow with the patient (3, 4).

To achieve tissue regeneration, cell-based therapies, tissue-inducing factors, and biocompatible scaffolds are usually investigated singularly or in combination (5-18).

In 1893, Barth (19) first introduced the concept of “scaffolding” as a porous matrix or an implant in which cells can infiltrate and regenerate the local tissue. In the last two decades, this concept has been extended to indicate natural and synthetic substrates that can temporarily support cells (20, 21) and direct their fate towards cell-material interactions and the release of biological factors (22-24). One of the most challenging aims is the design of scaffolds able to guide the process of tissue regeneration, and a basic principle involves growing cells in vitro into the three-dimensional (3D) organ or tissue. However, cells lack the ability to grow in favored 3D orientations and thus define the anatomical shape of the tissue. They randomly migrate to form a two-dimensional (2D) layer of cells. Since 3D tissues are required, cells have to be seeded onto porous matrices (ie scaffolds) to which the cells may attach and colonize (1, 3).

The scaffold therefore is a very important component for tissue engineering (10, 25, 26). The ideal scaffold should possess a repertoire of chemical, biochemical and biophysical cues able to control and to promote specific events at the cellular and tissue level (10).

A scaffold has to possess several crucial requirements (25, 26). It should be characterized by interconnecting pores of the appropriate scale in order to promote tissue integration and vascularization, and be made from material with biodegradability or bioresorbability controlled. It should have appropriate surface chemistry to facilitate cell attachment, differentiation and proliferation, and adequate mechanical properties to match the intended site of implantation and handling. Moreover, the scaffold should not induce any adverse response, and be easily manufactured into a variety of shapes and sizes. Therefore, several materials have been adopted or synthesized to realize scaffolds.

Over the last two decades, the concept of cell guidance in tissue regeneration has also been extensively discussed and progressively revised as new knowledge of the complex features of cell-material interaction have been disclosed (10-27). A major part of this evolution can also be related to the development of novel scaffold materials, compatible with the cell guidance concept and resulting from contemporary advances in the fields of materials science and molecular biology (28). However, according to the requirements that scaffolds have to satisfy, these porous structures have been produced in several
ways, both by using conventional fabrication methods and more advanced methods (i.e., rapid prototyping (RP) techniques).

In this review, RP methods to manufacture scaffolds for tissue engineering will be discussed with a special focus on the basic principles and potential of the 3D fiber deposition technique.

**Rapid Prototyping Techniques for Scaffolds Manufacturing**

Materials of natural, synthetic, semi-synthetic, and hybrid origins have been proposed and tested as scaffolds for tissue regeneration (28-30).

Synthetic and natural inorganic ceramic materials, such as hydroxyapatite and tricalcium phosphate, have been considered as candidates for scaffold materials for bone tissue engineering (3, 31). These ceramics resemble the natural inorganic component of bone and possess osteoconductive properties (3, 32). The main drawback is that they are inherently brittle and cannot match the mechanical properties of bone. Moreover, ceramic scaffolds are not suitable for the growth of soft tissues since they are characterized by different cellular receptors and mechanical performances. Synthetic and natural polymers are an attractive alternative and versatile in their applications to the growth of most tissues (33).

As for synthetic polymers, aliphatic polyesters such as polyglycolic acid (PGA), polylactic acid (PLLA), their copolymers (e.g., PLGA) and polycaprolactone (PCL) are the most commonly used polymers for tissue engineering scaffold applications (10, 25, 34).

Products obtained from the degradation of these polymers (glycolic acid and lactic acid) are present in the human body and can be removed by natural metabolic pathways. On the other hand, naturally derived protein or carbohydrate polymers have been considered as scaffold materials for the growth of several tissue types (10, 29). By far the most popular natural polymer used for tissue engineering scaffolds is collagen.

Different techniques have been developed to fabricate 3D porous scaffolds, each characterized by its own advantages and limitations. The introduction of RP technologies in the biomedical field has led to the division of scaffold fabrication techniques into two groups, defined as “conventional” and “novel” methods (3, 35). In particular, conventional methods are defined as processes to obtain scaffolds that are characterized by continuous, uninterrupted pore structure, however, lacking any long-range channeling microarchitectural. Basically, these techniques include fiber meshes/fiber bonding, gas foaming, solvent casting/particulate leaching, phase separation, melt molding, freeze drying, solution casting, and emulsion freeze drying (3, 35).

The internal architecture of scaffolds, including pore size, pore shape and interconnectivity, are critical to their in vivo and mechanical performances, since it influences the degree and the path of tissue regeneration, and determines the mechanical properties of the scaffolds (36-44). Fortunately, in many scaffold manufacturing techniques, the control of the internal architecture and interconnectivity is limited (36, 45). Conventional scaffold processing techniques are, in fact, incapable of precisely controlling pore size, pore geometry, spatial distribution of pores and construction of internal channels within the scaffold (3, 35). For example, scaffolds produced by solvent casting/particulate leaching cannot guarantee interconnection of pores because this is dependent on whether the adjacent salt particles are in contact. Furthermore, the interconnectedness provided by these techniques is strongly related to many processing variables, such as the rate of solvent evaporation and the 3D contact between the porogen particles (36, 46). Consequently, using conventional scaffold manufacturing methods to have precise control over the internal architecture and interconnectivity is very difficult. Moreover, scaffolds fabricated with conventional techniques can be shaped with custom-made molds.

Conversely, the technology transfer of solid freeform fabrication (SFF) to tissue engineering represents the key to produce customized scaffolds with reproducible internal morphology. This allows for a higher degree of architectural control, making structures to increase the mass transport of oxygen and nutrients throughout the scaffold (3, 35).

SFF is a collective term for a group of technologies that can manufacture objects in a layer-by-layer fashion from the 3D computer design of the object. SFF was initially developed for fabricating prototype engineering parts, thus the name “rapid prototyping” (RP) is also widely used (3, 35, 36, 47-49). Since 1987 more than 20 SFF technologies have been developed and these technologies differentiate themselves mainly by the method by which the layers are laid down, solidified, and attached to the previous ones (3, 35, 36, 47-49).

Even though there are several commercial variants of SFF technology that differ significantly in the way they build up 3D models, they also present several common features since all SFF technologies are characterized by three basic steps in their process: data input, data file preparation, and object building (3, 36, 50, 51). In particular, the general process involves producing a computer-generated model using computer-aided design (CAD) software. Successively, a CAD model is expressed as a series of cross-sectional layers, and the data are implemented by the SFF machine that creates the physical model. Some SFF technologies require an additional step of post-processing to remove either temporary supports or the excessive material trapped inside the void space in the built structure. Furthermore, if a second type of data source is data obtained from computed tomography (CT) or magnetic resonance imaging (MRI) medical scans can be used to create a customized CAD model and, consequently, a scaffold which should be characterized by the exact external shape required to
correct the damaged tissue site (3, 36, 50, 51).

Among these SFF technologies, many have been modified or developed towards the manufacturing of tissue engineering scaffolds, including 3D printing, fused deposition modeling, ink-jet printing, stereolithography, selective laser sintering and a few other extrusion-based technologies, such as 3D Bioplotting (3, 35, 36, 52-57).

3D printing incorporates a technology to eject a binder from a jet head that moves in accordance with the CAD cross-sectional data, onto a polymer powder surface. The binder dissolves and joins adjacent powder particles. The piston chamber is lowered and refilled with another layer of powder and the process repeated. The unbound powder acts to support overhanging or unconnected features and needs to be removed after component completion (3, 54).

Fused deposition modeling uses a moving nozzle to extrude a fiber of polymeric material from which the physical model is built layer-by-layer. The model is lowered and the procedure repeated. Although the fiber must also produce external structures to support overhanging or unconnected features that need to be manually removed, the pore sizes in tissue engineering scaffolds are sufficiently small enough for the fiber strand to bridge across without additional support structures (3, 26, 53, 58).

In ink-jet printing the layout of the system consists of a build platform set on top of an elevator with a rolling cutter blade on one side of the platform and two print jets mounted on x, y rails. Two print materials are used, build materials and support materials. The build jet first lays down the design pattern by printing droplets onto the platform. The support jet then prints support material around the printed pattern. After printing, the cutter blade comes over and cuts the build layer to a predetermined layer thickness, thereby controlling the accuracy in the z-direction. The build jet then prints build material for the next layer. The process repeats itself until the entire object is completed (3, 35, 36).

As for stereolithography, the basic process involves selective polymerization of a liquid photocurable monomer by an ultraviolet laser beam. The UV beam is guided (x- and y-axis control) onto the liquid monomer surface in accordance with the CAD cross-sectional data. After the first layer is built, the elevator holding the model is lowered into the vat so as to allow the liquid photopolymer to cover the surface. A “wiper arm” is then displaced over the liquid to flatten the surface. The procedure is repeated until the model is completed. This system requires support structures to be added to the model, to prevent any overhanging or unconnected features from falling to the bottom of the liquid-filled vat. After completion, the model is raised and any support structures are removed manually (3, 35, 36, 52, 59).

In selective laser sintering, the build material of the system is either a polymer or a polymer-coated ceramic powder. The layout of the system consists of a build plat-
Rapid prototyped scaffolds for tissue regeneration

Among all of the RP techniques, 3D plotting (55-57) and 3D fiber deposition (62, 63) have been recently developed and used for tissue engineering purposes. In particular, 3D fiber deposition may be considered as a modified technique of 3D plotting for the extrusion of highly viscous polymers, and it is a fused deposition technique in which a molten polymer is extruded and then deposited through a servo-mechanically controlled syringe that applies pressure (Fig. 1) (61, 64, 65). This process allows the realization of scaffolds with specific shape and size and 100% interconnectivity. Such scaffolds possess a defined structure and architecture, and can be built with a customized shape by CAD/CAM techniques.

The key element of the 3D fiber deposition technique is a dispensing machine known as a Bioplotter, which was developed by Landers et al (55-57) to realize scaffolds from hydrogels for soft tissue engineering. In particular, it consists of a dispenser, equipped with a heating jacket that is movable in three dimensions. The basic process involves dispensing of a flowable material stored into a cartridge through a thin needle by air-pressure control, and its subsequent hardening. The material can be dispensed in the presence of air or in a liquid. The advantage of dispensing in a liquid medium is that the buoyancy in the liquid prevents deformation in the dispensed but not the completely hardened structure (Fig. 2) (56). Consequently, only highly viscous materials, such as polymer melts, can be processed in air. Hardening processes can be obtained through thermally induced solidification, solidification induced by a chemical reaction (e.g. a reactive component is added to the plotter material and a second one is added to the plotter medium), and solidification induced by precipitation. Among solidification processes, the thermally induced one includes solidification of melts and gelling of thermally reversible hydrogels, such as gelatin and agar.

The knowledge of the critical processing parameters is crucial to develop 3D fiber-deposited scaffolds. Briefly, if a molten polymer is assumed as a viscous Newtonian fluid and the Hagen-Poiseuille equation as valid (62, 66), the flow rate from the nozzle can be expressed according to:

$$Q = \frac{\pi \Delta P d^4}{128 \eta}$$  \[1\]

The above described Hagen-Poiseuille equation 1 indicates that the flow rate (Q) is directly proportional to both the pressure gradient (\(\Delta P\)) across the syringe and needle tip, and the needle diameter (d). Moreover, Q is inversely proportional to needle length (l) and polymer viscosity (\(\eta\)). A high Q value may result in over-deposition of the fiber, thus reducing porosity, whilst a low Q value reduces the fiber diameter, compromising the overall scaffold integrity.

A decrease in needle diameter reduces the flow rate, requiring considerably greater pressures to extrude fibers, and in the case of small needle diameters the pressures required to achieve a suitable flow rate can be greater.

### Table I - Comparison of Different Rapid Prototyping (RP) Techniques on the Basis of Materials, Advantages and Disadvantages (35, 56)

<table>
<thead>
<tr>
<th>RP techniques</th>
<th>Materials</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stereolithography</td>
<td>Reactive resins</td>
<td>Good mechanical strength</td>
<td>Limited to reactive resins (mostly toxic)</td>
</tr>
<tr>
<td>3D Printing</td>
<td>Ink + powder of bulk polymers, ceramics</td>
<td>Easy to remove support materials</td>
<td>Limited choice of photopolymerizable and biocompatible liquid polymer materials</td>
</tr>
<tr>
<td>Inkjet Printing</td>
<td>Wax or wax compounds</td>
<td>No inherent toxic components</td>
<td>Weak bonding between powder particles</td>
</tr>
<tr>
<td>FDM/FDC</td>
<td>Some thermoplastic polymers/ceramics</td>
<td>Fast processing</td>
<td>Rough surface</td>
</tr>
<tr>
<td>Selective Laser Sintering</td>
<td>Metals, ceramics, bulk polymers, compounds</td>
<td>Low costs</td>
<td>Post-processing</td>
</tr>
<tr>
<td>3D Bioplotting</td>
<td>Swollen polymers (hydrogels), thermoplastic polymers, reactive resins, ceramics</td>
<td>Excellent accuracy</td>
<td>Slow process</td>
</tr>
</tbody>
</table>

**TABLE I - COMPARISON OF DIFFERENT RAPID PROTOTYPING (RP) TECHNIQUES ON THE BASIS OF MATERIALS, ADVANTAGES AND DISADVANTAGES (35, 56)**
than those usually used in practice, thus needing changes in viscosity (62). Even though for small needle diameters polymer viscosity can be reduced through the addition of specific solvents or increasing the syringe temperature, the incomplete removal of solvents post-processing or polymer exposure to high temperatures can be detrimental to scaffold biocompatibility (48, 55). By exploiting the knowledge about the plotting process and the properties of the materials, 3D fiber-deposited scaffolds with desired properties may be obtained.

For the first time, Lander et al (55-57) realized and characterized hydrogel scaffolds with a desired external shape and a well defined internal pore structure through 3D Bioplotting, also suggesting this technology as a biofunctional and cell compatible processing for hydrogels in the area of RP techniques. In particular, the versatile application potential of rapid prototyped agar scaffolds coated with a mixture of hyaluronic and alginic acid or with fibrin was demonstrated in cell culture using two cell types which were seeded on these hydrogel scaffolds, a human osteosarcoma cell line (CAL-72) and a mouse connective tissue fibroblast (55-57).

Woodfield et al (62) presented and characterized the 3D fiber deposition technique for making 3D poly(ethylene glycol)-terephthalate-poly(butylene terephthalate) (PEGT/PBT) block co-polymer scaffolds with a 100% interconnecting pore network for articular cartilage tissue engineering. This technique allowed to “design-in” desired scaffold characteristics layer-by-layer by accurately controlling the deposition of molten co-polymer fiber from a pressure-driven syringe placed onto the mobile arm of a 3D plotter. Values of dynamic stiffness similar to those of native articular cartilage explants were obtained by suitably varying porosity, pore geometry and PEGT/PBT composition. It was demonstrated that these 3D fiber-deposited scaffolds seeded with bovine articular chondrocytes supported a homogeneous cell distribution and subsequent cartilage-like tissue formation following in vitro culture as well as subcutaneous implantation in nude mice. These results were highlighted by the presence of articular cartilage extracellular matrix constituents - glycosaminoglycans and type II collagen - throughout the interconnected pore structure. Interesting results were also achieved with respect to the attachment of expanded human articular chondrocytes (62).

Since nutrient limitation (e.g. oxygen) has been considered as a cause of the onset of chondrogenesis solely within the peripheral boundaries of larger constructs, the effect of the 3D fiber-deposited PEGT/PBT scaffold architecture on oxygen gradients in tissue engineered cartilaginous constructs was assessed by Malda et al (63) through...
Rapid prototyped scaffolds for tissue regeneration

Microelectrode measurements, and then compared to the results obtained from a compression-molded and particle-leached scaffold.

Even though it was not observed, an effect of scaffold architecture on oxygen gradients, cell distribution and matrix deposition were enhanced in 3D fiber-deposited scaffolds if compared to the compression-molded and particle-leached ones (63). All of these results stressed the importance of a rationally designed scaffold for cartilage tissue engineering applications, and suggested that organized structures, such as the 3D fiber-deposited scaffolds, with their less tortuous and more open structure may offer possibilities for the regulation of nutrient supply (63).

In this context, Moroni et al (61, 64, 65) designed, manufactured and characterized 3D fiber-deposited scaffolds processing PEOT/PBT block copolymers which belong to a class of materials known as thermoplastic elastomers, and possess mechanical properties depending on the PEOT/PBT weight ratio in block form and on the molecular weight of the initial poly(ethylene glycol) (PEG) blocks.

Several PEOT/PBT copolymer compositions were used to fabricate scaffolds with a Bioplotter device through heating polymer granules. Moreover, pores were varied in shape and size, by changing fiber diameter, spacing, sequence of stacking (i.e. pattern), and layer thickness (61, 64, 65). However, since pore geometry (and, hence, porosity) is defined by fiber diameter and spacing, and layer thickness, it is also strongly dependent on the deposition speed used during the process (Fig. 3) (64).

Accordingly, in order to assess the influence of the pores geometry and architecture on the mechanical performances, 3D fiber-deposited PEOT/PBT scaffolds were characterized through dynamic-mechanical analysis (DMA).

In particular, with increasing porosity, DMA analysis showed a decrease of the elastic properties such as the storage modulus ($E'$) (Fig. 4a) (65), whilst an increase of the modulus was evaluated with decreasing the fiber spacing (Fig. 4b) (64). Furthermore, it was also evidenced that pore geometry (and, hence, porosity) is defined by fiber diameter and spacing, and layer thickness, it is also strongly dependent on the deposition speed used during the process (Fig. 3) (64).

Accordingly, in order to assess the influence of the pores geometry and architecture on the mechanical performances, 3D fiber-deposited PEOT/PBT scaffolds were characterized through dynamic-mechanical analysis (DMA).

In particular, with increasing porosity, DMA analysis showed a decrease of the elastic properties such as the storage modulus ($E'$) (Fig. 4a) (65), whilst an increase of the modulus was evaluated with decreasing the fiber spacing (Fig. 4b) (64). Furthermore, it was also evidenced that pore geometry (and, hence, porosity) is defined by fiber diameter and spacing, and layer thickness, it is also strongly dependent on the deposition speed used during the process (Fig. 3) (64).

![Fig. 3 - Effect of deposition speed on scaffold porosity and fiber diameter. Results obtained from 3D fiber-deposited PEOT/PBT scaffolds with specific composition and architecture (64).](image)

![Fig. 4 - a) Effect of porosity on the storage modulus $E'$ for 3D fiber deposited PEOT/PBT scaffolds with specific composition (65); b) Effect of fiber spacing on the storage modulus $E'$ for 3D fiber deposited PEOT/PBT scaffolds with specific composition and architecture, considering two different fiber diameters (64); c) Effect of architecture on the storage modulus $E'$ for 3D fiber deposited PEOT/PBT scaffolds with same composition and porosity (65).](image)
that for PEOT/PBT scaffolds with the same composition and porosity but different architectures, $E'$ varied within a wide range of values (Fig. 4c) (65).

Another interesting approach was to make hollow fibers directly integrated in a 3D fiber-deposited structure, thus realizing scaffolds which can be used in tissue engineering and controlled drug delivery applications as possible smart biomaterial devices (67).

To realize hollow fibers with controllable hollow cavity diameter and shell thickness a rheological phenomenon, which is known as “viscous encapsulation” and often undesired in molten polymeric blends, was considered (67).

Briefly, when two components of a polymer blend possess a significant difference in viscosity in the molten state fibers with a shell-core configuration can be extruded. The polymer with lower viscosity tends to shift, when flowing through a narrow duct, such as the nozzle of an extruder (e.g., the needle used during the 3D Bioplotting process), towards the walls of the nozzle during extrusion. Due to the higher shear stresses at the walls this separation of the components produces a stratification or a “canalization” effect, thus providing fibers with a shell-core structure (Fig. 5). By removing the core polymer by selective dissolution, hollow fibers can be obtained (67).

Taking into consideration this phenomenon, Moroni et al (67) manufactured and characterized PEOT/PBT scaffolds with hollow fibers through the direct deposition of the viscous encapsulated fibers in a CAD/CAM fashion and the subsequent selective core dissolution. In particular, PEOT/PBT scaffolds with hollow fibers were obtained by soaking 3D shell-core scaffolds in a specific solvent (i.e., acetone) for the poly(butylmethacrylate-methylmethacrylate) (P(BMA/MMA)) or for the PCL core polymers. Consequently, P(BMA/MMA) or PCL was selectively dissolved and only the PEOT/PBT well-organized structure was left. However, it was found that viscous encapsulation occurred for specific values of melting index ratios when these polymers are extruded under proper rheological conditions of the 3D fiber deposition process used (67).

Accordingly, by varying the polymers in the blend, the blend composition, and the extrusion needle diameter, the possibility to control the hollow cavity diameter and the shell thickness was also highlighted (67).

Benefiting from the same principle, biphasic 3D fiber-deposited scaffolds for cartilage tissue engineering with a shell-core fiber structure, in which the core polymer provided appropriate mechanical properties and the shell polymer acted as a coating characterized by specific physicochemical surface properties, were designed and studied (68). In this case, biphasic shell-core PEOT/PBT 3D scaffolds were manufactured from PEOT/PBT copolymers with different compositions (hence, a different melting index), by exploiting viscous encapsulation and 3D fiber deposition technique. If compared to the core polymer, the shell polymer contained a higher molecular weight of the initial PEG segments used in the copolymerization and a higher weight percentage of the PEOT domains. Rapid prototyped scaffolds entirely produced with the shell or with the core polymers were also characterized and the results were compared with those of biphasic shell-core scaffolds. Even though for all of the investigated scaffolds comparable amounts of entrapped chondrocytes and of extracellular matrix formation were obtained, chondrocytes maintained their rounded shape and aggregated during the culture period on shell-core 3D fiber-deposited scaffolds, thus suggesting a proper cell...
Rapid prototyped scaffolds for tissue regeneration

differentiation into articular cartilage. Moreover, from a mechanical point of view the biphasic shell-core scaffolds also evidenced an improved dynamic stiffness. All of these results suggested that the use of these biphasic shell-core 3D fiber-deposited scaffolds with appropriate mechanical and surface properties is a promising solution for cartilage tissue engineering (68).

Furthermore, since cell seeding efficiency still remains a critical factor for optimal tissue regeneration, the possibility to combine the 3D fiber deposition technique with electrospinning was also demonstrated (69); therefore, obtaining scaffolds where the periodical macrofibers typical of 3D fiber-deposited structures were integrated with the random electrospun ones. In these integrated structures, the 3D fiber-deposited scaffold acts as a structural support with adequate mechanical properties, whilst the electrospun network mainly works as a cell entrapment system (69).

An additional challenge in tissue engineering is that most tissues and organs are multiphasic in nature and contain multiple cell types. Consequently, an ideal scaffold should be capable of supporting multilineage cell types and few attempts have been made to engineer tissues consisting of different cell types (70-72).

For these reasons, stabilized osteoblast-like cells (MG63) and normal endothelial cells (human umbilical vein endothelial cells, HUVEC) were co-seeded onto 3D fiber-deposited PCL scaffolds, and cultured by means of a rotary cell culture system in order to study their reciprocal cell interactions for enhanced bone tissue engineering (70).

The proposed co-cultural endothelial and osteoblast-like cell model is based on the close mutual interaction of the two cell types, and this is sustained by histological evidence that osteoblasts and osteoprogenitor cells are always located adjacent to blood vessel endothelial cells (70, 73, 74). Moreover, with regard to embryonic skeletal tissue osteogenesis and angiogenesis are temporally related (70, 75). All of these in vivo findings clearly highlight that these processes are mutually interdepen-
dent (70, 76) and that endothelial cells may accelerate bone formation through angiogenesis as well as in bone remodeling (70, 77).

Since the maintenance, survival and growth of a 3D bio-construct is strongly related to a delicate balance between cell metabolism, nutrient transport and scaffold properties (70, 78, 79), porous yet sufficiently stiff 3D structures with a suitable architecture were manufactured.

In particular, 3D fiber-deposited PCL scaffolds were fabricated with a Bioplotter device by extruding the molten polymer and alternatively depositing the fibers along the 0° and the 90° directions between two successive layers, thus obtaining a 0°/90° pattern (70).

As for the mechanical properties of these 3D PCL scaffolds, compression tests highlighted a modulus of 134.6 ± 8.5 MPa and a stress-strain curve (Fig. 6) (70) similar to that of a flexible foam (80).

After an initial relatively stiff mechanical response, there is a region with lower stiffness, finally followed by another stiff portion, similar to the densification region of flexible foams. However, unlike the typical behavior of flexible foam, the central part of the curve does not show a plateau (70). MicroCT and imaging analyses (Fig. 7) confirmed that the 3D fiber-deposited PCL scaffolds were characterized by a precise pore size and a repeatable microstructure, also showing sufficient consistency between real and theoretical values and an interconnectivity of 100% (70).

In such a study on dynamic co-seeding onto 3D fiber-deposited PCL scaffolds, Kyriakidou et al (70) underlined how osteoblasts increase proliferation of endothelial cells and endothelial cells amplify the growth of osteoblasts but decrease their differentiation. It was also suggested that dynamic seeding of osteoblasts and endothelial cells onto a 3D fiber-deposited polymeric scaffold was a useful approach to study the mechanisms of the interaction of endothelial and osteoblast cells, and to achieve a functional...
hybrid in which angiogenesis, furnished by neo-vascular organization of endothelial cells, may further support osteoblast growth (70).

In light of what has been said, 3D fiber deposition is a powerful technique to realize multifunctional and tailor-made scaffolds with suitable mechanical and surface properties; therefore, satisfying the need for tissue engineered scaffolds with an organized and repeatable microstructure, which enables cells to assemble in an ordered matrix and allows adequate nutrient perfusion.

CONCLUSION

Tissue engineering is going to be successful in replacing or repairing damaged tissues, and optimizing the scaffold is a crucial point. It appears clear that a scaffold has to be characterized by the correct balance between architectural features, porosity, physicochemical and biological properties.

RP techniques have the potential to optimize the scaffolds since they overcome limitations related to conventional fabrication methods, creating porous structures with controlled internal microarchitecture. Among the several RP methods, the 3D fiber deposition technique has emerged as a means to realize well defined and custom-made scaffolds for tissue regeneration, with 100% interconnected pores, also because of its flexibility to process a wide range of materials.

The mechanical behavior of the 3D fiber-deposited scaffolds is dependent not only on the intrinsic properties of the material processed, but also on the different 3D architectural and geometric features, thus highlighting the possibility to tailor their mechanical properties suitably. Consequently, if mimicking the biomechanical behavior of the tissue to be grown is a key point in tissue engineering, the 3D fiber deposition technique may be considered as a powerful tool to create scaffolds for specific applications.

Moreover, hollow fibers directly integrated in a rapid prototyped structure, as well as biphasic shell-core polymeric 3D scaffolds where cell and tissue compatibility requirements are combined with mechanical requisites, can also be obtained by combining the “viscous encapsulation” rheological phenomenon with the 3D fiber deposition technique.

On the other hand, in the engineering of multiphasic or multicomponent tissue constructs the dynamic co-seeding of two different cell lines onto a 3D fiber-deposited polymeric scaffold may represent a useful approach to investigate the mechanisms of the reciprocal cell interactions.

In conclusion, considering that great progress has been made in the field of RP techniques, a lot of research interest is being driven towards the development of 3D fiber-deposited scaffolds functionalized with appropriate biomolecules; therefore, obtaining bioactive and morphologically controlled structures with tailored mechanical and surface properties to be used as smart biomaterial devices in tissue engineering applications.

Conflict of interest statement: None.

Address for correspondence:
Antonio Gloria
Institute of Composite and Biomedical Materials
National Research Council
P.le Tecchio 80
80125 Naples
Italy
angloria@unina.it

REFERENCES

Rapid prototyped scaffolds for tissue regeneration


