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Scaffold design and fabrication technologies for engineering tissues — state of the art and future perspectives

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Abstract—Today, tissue engineers are attempting to engineer virtually every human tissue. Potential tissue-engineered products include cartilage, bone, heart valves, nerves, muscle, bladder, liver, etc. Tissue engineering techniques generally require the use of a porous scaffold, which serves as a three-dimensional template for initial cell attachment and subsequent tissue formation both *in vitro* and *in vivo*. The scaffold provides the necessary support for cells to attach, proliferate, and maintain their differentiated function. Its architecture defines the ultimate shape of the new grown soft or hard tissue. In the early days of tissue engineering, clinically established materials such as collagen and polyglycolide were primarily considered as the material of choice for scaffolds. The challenge for more advanced scaffold systems is to arrange cells/tissue in an appropriate 3D configuration and present molecular signals in an appropriate spatial and temporal fashion so that the individual cells will grow and form the desired tissue structures — and do so in a way that can be carried out reproducibly, economically, and on a large scale. This paper is not intended to provide a general review of tissue engineering, but specifically concentrate on the design and processing of synthetic polymeric scaffolds. The material properties and design requirements are discussed. An overview of the various fabrication techniques of scaffolds is presented, beginning with the basic and conventional techniques to the more recent, novel methods that combine both scaffold design and fabrication capabilities.

Key words: Tissue engineering; scaffolds; synthetic polymers; design and fabrication techniques.

1. INTRODUCTION

In the 1980s Bell *et al.* were among the first to tissue engineer bi-layered skin grafts. They showed that a collagen lattice seeded with autologous skin fibroblasts contracts and forms dermal tissue, and suspensions of epidermal cells applied to these lattices *in vitro* led to differentiation of the epidermal cells. This skin equivalent has been used clinically in the treatment of venous ulcers, acute wounds and split thickness donor sites. It was reported to have similar behavior to human

skin [1]. At present, tissue engineering techniques generally require the use of a porous scaffold, which serves as a three-dimensional specimen for initial cell attachment and subsequent tissue formation both *in vitro* and *in vivo*. Cells can be expanded in culture and seeded onto a scaffold that will slowly degrade and resorb, as the tissue structures grow *in vitro* and/or *in vivo* [2]. A number of materials, as well as scaffold design, have been experimentally and/or clinically studied. Ideally, a scaffold should have the following characteristics: (i) three-dimensional and highly porous with an interconnected pore network for cell/tissue growth and flow transport of nutrients and metabolic waste; (ii) biodegradable or bioresorbable with a controllable degradation and resorption rate to match cell/tissue growth *in vitro* and/or *in vivo*; (iii) suitable surface chemistry for cell attachment, proliferation, and differentiation; (iv) mechanical properties to match those of the tissues at the site of implantation; and (v) be easily processed to form a variety of shapes and sizes. FDA approved devices and implants made of polymers of synthetic origin, such as sutures, meshes etc. were used in the early days of tissue engineering [3]. Later, techniques were developed based on either heating macromolecules or dissolving them in a suitable organic solvent. In these scaffold fabrication techniques, the viscous behavior of the polymers above their glass transition or melting temperatures, and their solubility in various organic solvents were two important characteristics, which dictated the type of process, used. The aim of this paper is discuss the scaffold material and design characteristics, which are of specific interest to tissue engineers. The currently applied scaffold fabrication technologies will be evaluated with special emphasis on the solid free form fabrication methods.

2. SCAFFOLD MATERIAL

Today, four types of biomaterials have been experimentally and/or clinically studied as scaffold material for tissue engineering applications: (A) synthetic organic materials: aliphatic polyesters, polyethylene glycol; (B) synthetic inorganic materials: hydroxyapatite, tricalciumposphate, plaster of Paris, glass ceramics; (C) organic materials of natural origin: collagen, fibrin glue, hyaluronic acid; and (D) inorganic material of natural origin: coralline hydroxyapatite [3].

The focus of this review is on scaffolds, which are made of synthetic polymers. The meaning and definition of the words biodegradable, bioerodable, bioresorbable and bioabsorbable (Table 1) — which are often used misleadingly in the tissue engineering literature — are of importance to discuss the rationale, function as well as chemical and physical properties of polymer based scaffolds. In this paper the polymer properties are based on the definitions given by Vert *et al.* [4]. The first stage of tissue engineering begins with the design and fabrication of a porous 3D scaffold, the main topic of this review paper. In general, the scaffold should be fabricated from a highly biocompatible material, which does not have the potential to elicit an immunological, or clinically detectable primary or secondary for-

Table 1.
Definitions and terminology used in this review

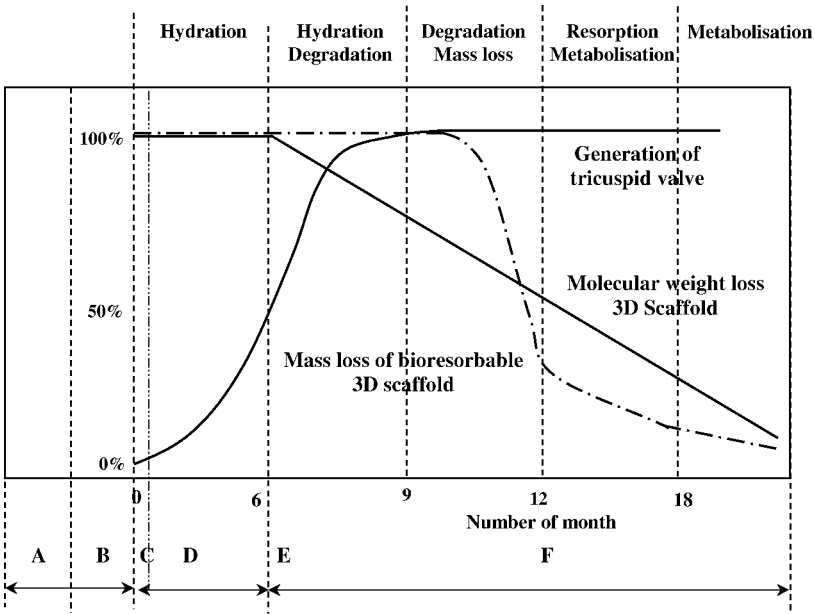
Biodegradable stands for solid polymeric materials and devices which break down due to macromolecular degradation with dispersion *in vivo* but no proof for elimination from the body (this definition excludes environmental, fungi or bacterial degradation). Biodegradable polymeric systems or devices can be attacked by biological elements so that the integrity of the system, and in some cases but not necessarily, of the macromolecules themselves, is affected and gives fragments or other degradation by-products. Such fragments can move away from their site of action but not necessarily from the body.
Bioresorbable stands for solid polymeric materials and devices which show degradation and further resorb *in vivo*; i.e. polymers which are eliminated through natural pathways either because of simple filtration of degradation by-products or after their metabolization. Bioresorption is thus a concept which reflects total elimination of the initial foreign material and of bulk degradation by-products (low molecular weight compounds) with no residual side effects. The use of the word 'bioresorbable' assumes that elimination is shown conclusively.
Bioerodable stands for solid polymeric materials or devices, which show surface degradation. Bioerosion is thus a phenomenon, which reflects the degradation, resorption and total elimination of the initially solid material via surface degradation by-products (low molecular weight compounds) produced without symptoms of residual side effects.
Bioabsorbable stands for solid polymeric materials or devices, which can dissolve in body fluids without any polymer chain cleavage or molecular mass decrease. For example, it is the case of slow dissolution of water-soluble implants in body fluids. A bioabsorbable polymer can be bioresorbable if the dispersed macromolecules are excreted.

eign body reaction. Furthermore, a polymer scaffold material has to be chosen that will degrade and resorb at a controlled rate. Currently, the design and fabrication of scaffolds in tissue engineering research is driven by three material categories: (I) biodegradable and bioresorbable polymers which have been used for clinically established products, such as collagen, hydrogels, polyglycolide (PGA), optically active and racemic polylactides (PLLA, P(DL)LA), polydioxanone (PDS), polycaprolactone (PCL), etc.; (II) polymers which are under clinical investigation for regulatory approval, such as polyorthoester (POE), polyanhydrides, polyhydroxyalkanoate (PHA), hyaluronic acid derivatives; and (III) the synthesis of entrepreneurial polymeric biomaterials, such as poly (lactic acid-co-lysine) etc., which can selectively bond specific cell phenotypes and guide the differentiation and proliferation into the targeted functional premature and/or mature tissue.

The application of a polymeric scaffold presents challenges and opportunities for a polymer chemists in a tissue engineering team from both material properties and processing. The polymer selection from a material science point of view is based on two different strategies with regard to the overall function of the scaffold.

Strategy I

In the first strategy (Fig. 1), the physical scaffold structure supports the polymer/cell/tissue construct from the time of cell seeding up to the point where the tissue



A - Fabrication of bioresorbable 3D scaffold.

B - Harvest cells from patient.

C - Cell seeding into a 3D scaffold in a static culture (petri dish).

D – Growth of mature tissue in a physiologic environment (bioreactor).

E - Surgical transplantation.

F - Implant adaptation and assimilation.

Figure 1. Tissue engineering a heart valve transplant via strategy (I). Graphical illustration of the complex interdependence of molecular weight loss and mass loss of the 3D scaffold matrix and time frame for cell/tissue generation.

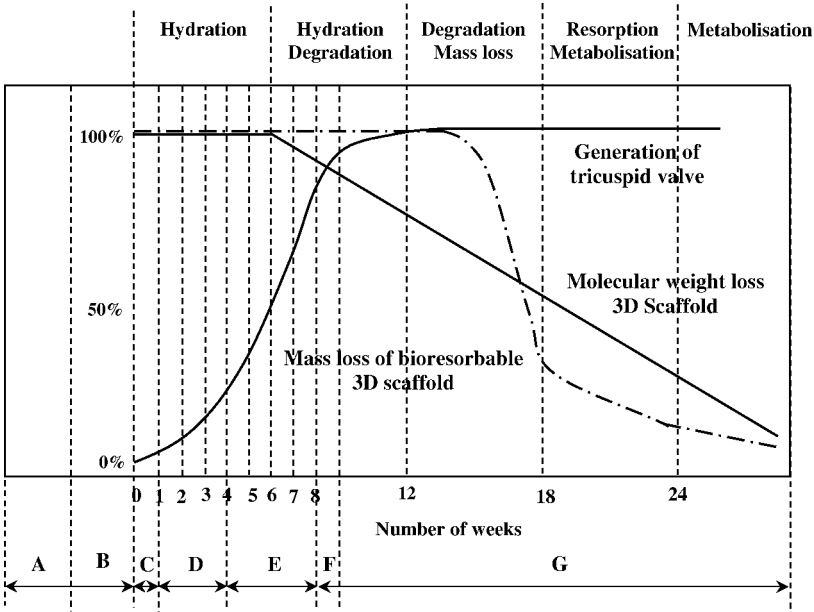
transplant is remodeled by the host tissue. In the case of tissue which are subjected to stress and strain, e.g. arteries and heart valves, the scaffold matrix must serve an additional function; it must provide sufficient temporary mechanical support to withstand *in vivo* stresses and loading. In Strategy I research programs, the material must be selected and/or designed with a degradation and resorption rate such that the strength of the scaffold is retained until the tissue engineered transplant is fully accommodated by the host tissue and can assume its structural role.

For example, multi-layered heart valve tissue is able to remodel *in vivo* under physiological loading [5–8]. It is a prerequisite that the degradation and resorption kinetics have to be controlled in such a way that the scaffold matrix retains its physical properties for a sufficient period of time. Thereafter, it will start losing its mechanical properties and should be metabolized by the body without a detectable

foreign body reaction (Fig. 1). The mechanical properties of the bioresorbable 3D scaffold/tissue construct at the time of implantation should match that of the host tissue as closely as possible. A tissue engineered heart valve construct should be sufficiently pliable to open with minimal opening pressures but with adequate strength and stiffness to function for a period until myocardial and endothelial tissue ingrowth can maintain the integrity by replacement of the slowly vanishing scaffold matrix. The degradation and resorption of the scaffold matrix would thus confer to the implanted valve a similar compliance to the surrounding host tissue, thereby eliminating potential stress discontinuities across the host tissue-implanted tissue interface. Today, no heart valve has been tissue engineered via strategy I due to the lack of a polymeric material with sufficient flexural and tensile strength. In contrast, scaffold/osteoblasts constructs have been placed *in situ* for bone regeneration because the scaffold matrix undergoes mainly compression loading in hard tissue defects. Polymers, such as PLA/PGA, PLA/PCL, and PCL can be processed into scaffolds, which have similar compression strength and modulus as cancellous bone.

Strategy II

For the second strategy (Fig. 2), the intrinsic mechanical properties of the scaffold architecture templates the cell proliferation and differentiation only within the *in vitro* phase. The degradation and resorption kinetics of the scaffold are designed to allow the seeded cells to attach, proliferate and secrete extracellular matrix in the static and/or dynamic growth phase. The physical support by the 3D scaffold is maintained until the cells have produced *in vitro* a premature tissue-structure that has sufficient mechanical integrity to support itself. Then, the polymer scaffold matrix gradually vanishes and the resulting space will be filled by new cell/tissue growth. Natural and synthetic polymers, such as collagen [6, 9], hyaluronan-gelatin [10], PGA [2, 5, 11–14], and PGA/PLA 90/10 [15–17] which have degradation and resorption kinetics of 2–4 months were used to engineer a number of tissues via strategy II. The restriction of that concept lies in the poor mechanical properties of the engineered tissue. For example, tissue engineered heart valves have been transplanted in the pulmonary position where only a minor physiological load is applied in comparison to the aortic position [5, 6]. Therefore, a number of researchers have started to engineer tissue in systems, such as bioreactors which mimic the physiological environment. A fluid-dynamic microenvironment provided by a bioreactor can mimic the different fluid conditions. Ma and Langer [18] showed that cartilage which was cultured for seven month in a bioreactor reached 40% of the mechanical properties of natural cartilage. In conclusion, dynamic systems permit *in vitro* culture of larger and better-organised 3D cell communities than can be achieved using static tissue culture techniques [19].



A - Fabrication of bioresorbable 3D scaffold.

B - Harvest cells from patient.

C - Cell seeding into a 3D scaffold in a static culture (petri dish).

D - Cell proliferation and differentiation in a dynamic environment (spinner flask).

E – Growth of mature tissue in a physiologic environment (bioreactor).

F - Surgical transplantation.

G - Implant adaptation and assimilation.

Figure 2. Tissue engineering a heart valve transplant via strategy (II). Graphical illustration of the complex interdependence of molecular weight loss and mass loss of the 3D scaffold matrix and time frame for cell/tissue generation.

3. SCAFFOLD DESIGN AND FABRICATION

A number of fabrication technologies have been applied to process biodegradable and bioresorbable materials into 3D polymeric scaffolds of high porosity and surface area [20, 21]. This part of the review will only discuss the gross morphological structure of scaffolds and not the surface topography which is a topic for a review itself. The conventional techniques for scaffold fabrication include textile technologies, solvent casting, particulate leaching, and membrane lamination and melt molding. From a scaffold design and function viewpoint each processing methodology has its pro and cons. It is the aim of this paper to aggregate the compiled information and to present this data in a comprehensive form.

Textiles

A number of textile technologies have the potential to be applied to design and fabricate highly porous scaffolds. Fibers provide a large surface area to volume ratio and are therefore desirable as scaffold matrix material. Yet, only non-woven constructs have been used. For example, promising results in tissue engineering bone, cartilage, heart valves, bladder, and liver have been achieved by using nonwoven composed of polymer fibers of PGA, PGA/PDLA, and PGA/PLLA. This work has been reviewed by Freed [22]. Textiles lack the structural stability to withstand biomechanical loading. Hence, different research groups have shown in a number of studies that felts made of PGA fibers and PGA/PLA 90/10 offers chemical and physical properties for executing strategy II. For improvement of mechanical properties a fiber bonding technique was developed to prepare interconnecting fiber networks with different shapes [23]. A composite material was thus produced consisting of non-bonded PGA fibers embedded in a PLLA matrix. The authors claim that the fibers are physically joined without any surface or bulk modification and retain their initial diameter. An alternative method of fiber bonding has been developed which involves coating a non-bonded mesh of PGA fibers with solutions of PLLA or PLGA [24]. A commercially available fleece uses a platen pressing process to three-dimensionally bond the PGA/PLA fibers with PDS fixation points. The degradation and resorption rate of the Ethisorb (Ethicon, Germany) is 2–3 months. Rotter *et al.* [15] studied both the Ethisorb and a PLLA fleece with degradation of 9–12 months. However, for load-bearing tissues such as bone and cartilage, the challenge for the cell/tissue construct is to have mechanical properties similar to those of the host tissue.

Cellular solids

The conventional techniques of scaffold fabrication: solvent leaching, gas foaming, vacuum drying, and thermally induced phase separation (TIPS) in combination with salt leaching produce foam-like structures which are generally classified in the engineering literature as cellular solids [25]. Various research groups have applied this technologies to fabricate scaffolds with a wide range of properties. However, there are numerous drawbacks to applying those scaffolds for tissue engineering applications. The pores are not fully inter-connected due to the formation of skin-layers during solvent evaporation. The pore size varies, as it is difficult to ensure that the porogens are well-dispersed and not agglomerated to form bigger particles. The thickness and length of the pore walls and edges vary depending on the solvent evaporation rate. The scaffolds cannot be made with thick sections as deeply embedded porogens become too distant from the surface and residual porogens may be left in the final structure. Use of organic solvents requires careful and complete removal of residual solvents (5 ppm) prior to clinical usage. It has been proposed to fabricate the scaffold by laminating membranes and introducing peptides and proteins layer by layer during the fabrication. Mikos *et al.* [26] fabricated porous

sheets in this way to form 3D structures. Chloroform was used as a bonding agent during the lamination process. The layering of porous sheets allows only a limited number of interconnected pore networks, and the mechanical properties of the resulting scaffold are insufficient. Solvent casted polymer–salt composites have also been extruded into a tubular geometry [27]. The disadvantages of the above technologies include: extensive use of highly toxic solvents, great time period required for solvent evaporation (days to weeks), labor intensive fabrication process, limitation to thin structures, residual particles in the polymer matrix, irregularly shaped pores, and insufficient interconnectivity.

The supercritical fluid-gassing process has been known for many years in the non-medical polymer industry [28] as well as in the pharmaceutical community [29]. This technology is used to produce foams and other highly porous products. The polymers, which can be used for this technology, have to have a high amorphous fraction. The polymer granules are plasticized due to the employment of a gas, such as nitrogen or carbon dioxide, at high pressures. The diffusion and dissolution of the gas into the polymer matrix results in a reduction of the viscosity, which allows the processing of the amorphous bioresorbable polyesters in a temperature range of 30–40°C [30]. The supercritical fluid-gassing technology allows the incorporation of heat sensitive pharmaceuticals and biological agents. However, on average only 10–30% of the pores are interconnected [31]. Harris *et al.* [32] combined this technology with particulate leaching to gain a highly interconnected void network. The researchers conclude that porosity and pore size can be controlled by varying the particle/polymer ratio and particle size.

Whang *et al.* [33, 34] developed a protocol for the fabrication of aliphatic polyester based scaffolds by using the emulsion freeze-drying method. Scaffolds with porosity greater than 90%, median pore sizes ranging from 15 to 35 μm with larger pores greater than 200 μm were fabricated. The scaffold pore architecture was highly interconnected, a feature which is necessary for tissue ingrowth and regeneration. Based on their results from an animal experiment, the interdisciplinary group proposed a scaffold design concept, which results in *in vivo* bone regeneration, based on hematoma stabilization [35]. The authors compare their *in vivo* bone engineering concept to the induction phase of fracture healing. The osteoprogenitor cells, which are in the blood of the osseous wound, are entrapped in the scaffold microarchitecture via the formation of a hematoma. The multipotent cells differentiate to osteoblasts due to the presence of growth factors, which are released by the host bone. However, the emulsion freeze-drying method is user and technique sensitive. The fabrication of a truly interconnecting pore structure depends on the processing method and parameters as well as on the used equipment.

Several groups [36–39] studied thermally induced phase separation technology to process polymeric 3D scaffolds. This technique has been used previously to fabricate synthetic membranes for non-medical applications. The method has been extensively applied in the field of drug delivery to fabricate microspheres, which allows the incorporation of pharmaceutical and biological agents, such

as bone morphogenetic proteins (BMPs) into the polymer matrix. In general, the micro- and macrostructure is controlled by varying the polymer material, polymer concentration, quenching temperature, and solvents. However, current research shows that the method, similar to emulsion freeze-drying technique, is user and technique sensitive and that the processing parameters have to be well controlled. Nam and Park [36] as well as Zhang and Ma [37] fabricated polymer and polymer/HA specimens with a porosity of up to 95%. At present, only pore sizes of up to 100 μm can be reproducibly fabricated by thermally induced phase separation technology.

A technique using ammonium bicarbonate salt particles was recently reported by Nam and co-workers [40]. The authors had reported successful fabrication of highly open porous PLLA scaffolds with well-interconnected pores of mean diameters 300–400 μm. Compressive moduli of 66–240 kPa were measured for porous scaffolds made of PLLA.

Solid free form (SFF) fabrication

There has been an increasing interest in the use of new techniques to design and fabricate scaffolds for tissue engineering. Advanced manufacturing technologies, also known as rapid prototyping or solid freeform fabrication technologies, are now being explored by investigators in such areas. These new techniques might become one of the most important tools for tissue engineering in the future. Rapid prototyping (RP) is the process of creating a three-dimensional (3D) object through repetitive deposition and processing of material layers using computer-controlled tools, based on 2D cross-sectional data obtained from slicing a computer-aided-design (CAD) model of the object. There are several RP systems developed such as stereolitography, selective laser sintering (SLS) laminated object manufacturing (LOM), three-dimensional printing (3-DP) and fused deposition modeling (FDM). For more than a decade now, RP is mainly used in the early verification of product designs and quick production of prototypes for form-fit testing in the manufacturing industries [41]. Medical researchers had also used this technology to produce artificial limbs, prosthetic implants, and surgical-planning models of internal body structures [42]. Data from MRI or CT scans of patients were often used for producing such models.

Conventional techniques do not allow tissue engineers to design and fabricate scaffolds with a completely interconnected pore network, highly regular and reproducible scaffold morphology, microstructure which varies across the scaffold matrix, and which is solvent-free, using a computer-controlled process. Such matrix architecture is advantageous in instances where tissue engineers want to grow a bi- or multiple tissue interfaces. Rapid prototyping technologies have the potential to design a 3D construct in a multi-layer design within the same gross architectural structure [43].

Three-dimensional printing

Three-dimensional printing (3-DP) is a solid-freeform fabrication process, which produces components by ink-jet printing a binder into sequential powder layers. It was developed at Massachusetts Institute of Technology [44, 45]. Firstly, a thin distribution of powder is spread over the surface of a powder bed. From a computer model of the part, a slicing algorithm computes information for the layer. Using technology similar to ink-jet printing, a binder material is ejected onto the powder where the object is to be formed. A piston then lowers so that the next layer of powder can be spread and selectively bonded. This layer by layer process repeats until the part is completed. The packing density of the powder particles has a profound impact on the results of the adhesive bonding, which in turn affects the mechanical properties of the build part. When the ink droplet impinges on the powder layer, it forms a spherical aggregate of binder and powder particles. Capillary forces will cause adjacent powder aggregates, including that of the previous layer, to merge. These layers will form locally a solid powder-based band, which finally will add up to build a solid model. The binding energy is composed of two components, one its surface energy and the other its kinetic energy.

Giordano *et al.* [46] studied the mechanical properties of 3D-printed PLLA parts. Test bars were fabricated from low and high molecular weight PLLA powders with chloroform as a binder. The binder printed per unit length of the powder was varied to analyze the effects of printing conditions on mechanical and physical properties of the PLLA bars. Cold isostatic pressing was also performed after printing to improve the mechanical properties of the printed bars. The maximum measured tensile strength for the low molecular weight PLLA (53 000) was 17.40 ± 0.71 MPa and for high molecular weight PLLA (312 000) was 15.94 ± 1.50 MPa. Kim *et al.* [47] evaluated the survival and function of hepatocytes on a scaffold with an intrinsic network of interconnected channels under continuous flow conditions. The scaffolds were designed and fabricated using the technique of 3-DP on copolymers of polylactide–coglycolide (PLGA 85 : 15). 3-DP was also used to selectively direct a solvent onto PLGA powder particles packed with sodium chloride particles (45–150 μm). The polymer scaffolds were fabricated in the shape of a cylinder 8 mm in diameter and 7 mm high. They contained twelve interconnected longitudinal channels (800 μm in diameter) running through the length of the scaffold and twenty-four interconnected radial channels (800 μm diameter) at various lengths of the devices. The salt crystals were leached out to yield porous devices of porosity 60% with micropores 45–150 μm in diameter. Park *et al.* [48] had also reported on the use of such 3D-fabrication technique in preparing patterned PLLA substrates to study the spatial organization of cells. They demonstrated that the scaffold surfaces could be made selectively adhesive for certain cell types by modifying the polymer surface to promote cell attachment. The 3-DP process is performed under room temperature conditions. Hence, this technology has great potential in tissue engineering applications because cells, growth factors, etc. can be incorporated into a porous scaffold without inactivation

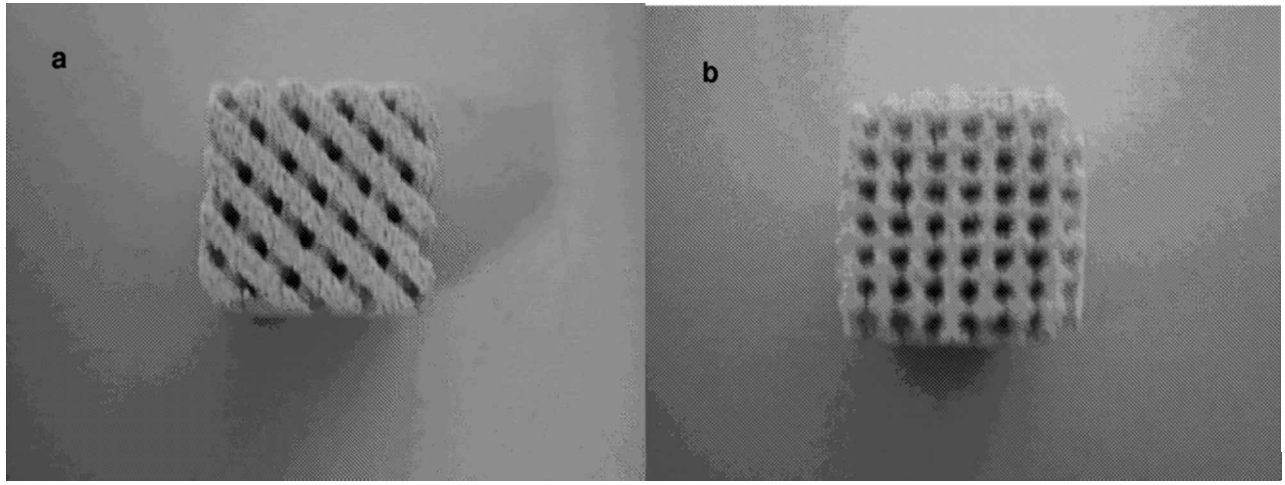


Figure 3. Scaffolds made of a starch/chitosan blend designed and fabricated via a 3D printing process. (This figure is published in colour on http://www.vsppub.com/jconts/JBS)

if non-toxic solvents, e.g. water based binders can be used [49]. Our group deigns and fabricates biodegradable scaffolds via 3-DP by using powder blends of starch/chitosan and starch/chitosan/hydroxyapatite (Fig. 3) [50, 51].

Fused deposition modeling

The FDM process forms 3D objects from a CAD file as well as digital data produced by an imaging source such as computer tomography (CT) or magnetic resonance imaging (MRI). The process begins with the design of a conceptual geometric model on a CAD workstation. The design is imported into software, which mathematically slices the conceptual model into horizontal layers. Toolpaths are generated before the data is downloaded to the FDM hardware. The FDM extrusion head operates in the X- and Y-axes while the platform lowers in the Z-axis for each new layer to form. In effect, the process draws the designed model (scaffold) one layer at a time [52].

Thermoplastic polymer filament feeds into the temperature-controlled FDM extrusion head where it is heated to a semi-liquid state. The head extrudes and deposits the material in ultra-thin layers onto a fixture-free base. The head directs the material precisely into place. The material solidifies, laminating to the preceding layer. Parts are fabricated in layers, where a layer is built by extruding a small bead of material, or road, in a particular lay-down pattern, such that the layer is covered with the adjacent roads. After a layer is completed, the height of the extrusion head is increased and the subsequent layers are built to construct the part. In the past, users could only use a few non-resorbable polymeric materials, such as polyamide, ABS, and other resins. At present, the author's multidisciplinary group has been able to evaluate the parameters to process PCL and PCL/HA by FDM [53]. Our results show that FDM allows to design and fabricate bioresorbable 3D scaffolds with a fully interconnected pore network. Due the computer-controlled processing the scaffold fabrication is highly reproducible. The mechanical properties and *in vitro* biocompatibility of polycaprolactone scaffolds with a porosity of 61% ±1 and two matrix architectures have been studied. The honeycomb-like pores had a size falling within the range of $360 \times 430 \times 620$ μm. The scaffolds with a $0/60/120^{\circ}$ lay-down pattern had compressive stiffness and 1% offset yield strength in air at 22°C of 41.9 ± 3.5 and 3.1 ± 0.1 MPa and in simulated physiological conditions 29.4 ± 4.0 and 2.3 ± 0.2 MPa, respectively. In comparison, the scaffolds with a $0/72/144/36/10^{\circ}$ lay-down pattern had compressive stiffness and 1% offset yield strength in air of 41.9 ± 3.5 and 3.1 ± 0.1 MPa and in simulated physiological conditions (saline solution at 37° C) 29.4 ± 4.0 and 2.3 ± 0.2 MPa, respectively. The obtained stress–strain curves for both scaffold architectures demonstrate the typical behavior of a honeycomb structure undergoing deformation. In vitro studies were conducted by using primary human fibroblasts and periosteal cells. Light, environmental scanning electron, and confocal laser microscopy as well as immunohistochemistry showed cell proliferation and extracellular matrix production on the PCL surface in the first culturing week [51]. Over a period of 3–4 weeks in culture, the

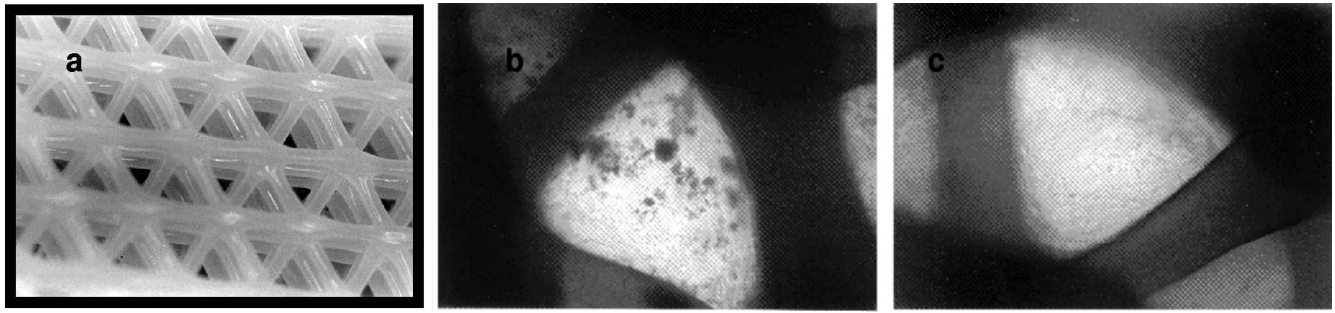


Figure 4. Light microscopical picture of a polycaprolactone scaffold with characteristics resulting from setting of different FDM build parameters for a 0/60/120° lay-down pattern (a). Inverted Light microscopy image (×100) of human bone marrow-derived stem cells (MSCs) cultured for three weeks in a medium which directs the MSCs into the osteoblastic lineage. The formation and mineralization of nodules viewed under phase contrast (b). Phase light contrast microscopy image (×100) of human chondrocytes seeded in a fibrin glue into a PCL scaffold with a 0/60/120° lay-down pattern. The PCL scaffold exhibited good biocompatibility evident in the increasing cell proliferation and cell activity. Microscopically, it was observed that the cells had attached and spread three-dimensionally in the fibrin glue and on the PCL bars. Chondrocytes did present the typical star-shaped morphology. (This figure is published in colour on http://www.vsppub.com/jconts/JBS)

fully interconnected scaffold architecture was completely three-dimensional filled by cellular tissue (Fig. 4).

Other rapid prototyping technologies

Landers and Muellhaupt [54] developed a versatile computer-guided manufacturing system which allows to design and fabricate scaffolds using hotmelts, solutions, pastes and dispersions of polymers, as well as monomers and reactive oligomers without requiring post-processing treatments. The system has a resolution of 200 μm. The basic principle of the multiphase jet solidification (MJS) process is to extrude a melted material through a jet. Koch and co-workers [55] reported the use of the MJS method to build scaffolds made of poly (D, L)-lactide. The material was first melted in the process chamber of MJS and extruded through a x-y-z-controlled jet. Special structures were designed to tissue engineer bone and cartilage. The structures had a reported pore size of 300–400 μm. Calvert and co-workers [56] developed an extrusion freeform fabrication method based on extrusion and deposition of viscous slurry through a fine needle. The stepper-motor driven syringe was mounted above a $x-y$ table, and both were controlled from a computer. According to the authors, their system had a resolution of about 0.5 mm with the typical layer heights being 0.2–1.0 mm and each layer took about 1 min to write. Both cross-linked polyacrylamide and agarose gels were fabricated using this method. Another RP technology reported was named shape deposition manufacturing (SDM). Marra *et al.* [57] reported the use of this method to construct osteogenic scaffolds based on blends of PCL and P(DL)LAGA incorporated with hydroxyapatite granules for bone tissue engineering applications. However, the authors did not describe the RP process in great detail. Furthermore, the utilization of a salt leaching process suggested that the authors were still relying on salt particles to produce the necessary micropores. The necessity of a complex 3D scaffold structure as the basic template for engineering tissue has encouraged our group to apply a micro-assembly manufacturing technology for scaffold fabrication (Fig. 5) [58]. The design and fabrication concept is based on joining micro-building blocks made of a bioresorbable polymer in order to create a scaffold with the desired chemical and physical properties.

4. CONCLUSIONS

Tissue engineering is set to evolutionize the treatment of patients and contribute significantly to life sciences in the next millennium. It is based on the concept that cells seeded onto 3D bioresorbable scaffolds can build native tissues under suitable *in vitro* and *in vivo* conditions. The use of regulatory approved synthetic polymers for the fabrication of scaffolds supports the drive for the clinical application of tissue engineering, however, a number of novel scaffold materials have been developed and are under investigation. Ideally, a scaffold material should permit

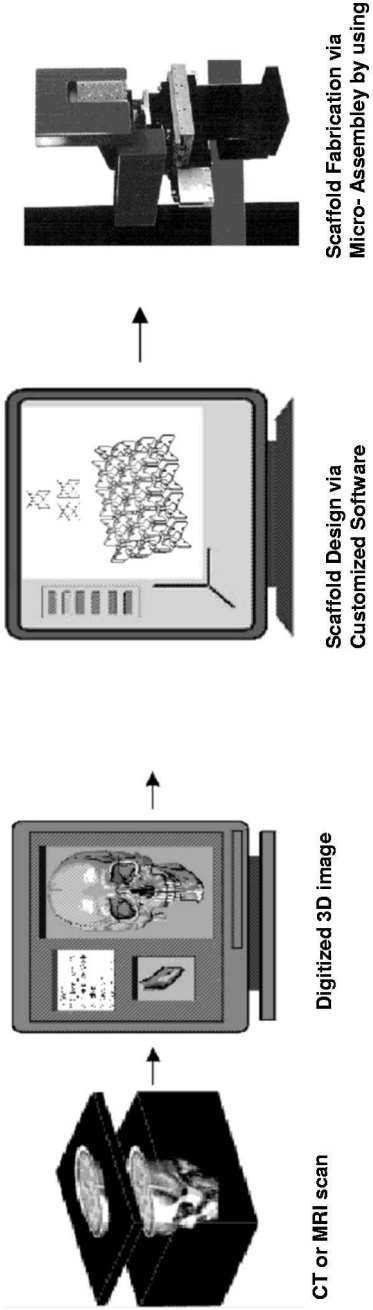


Figure 5. Graphical illustration of a concept to build a customized 3D scaffold via robot supported micro assembly. (This figure is published in colour on http://www.vsppub.com/jconts/JBS)

the application of a solid free form fabrication technology, so that a porous scaffold with any desired three-dimensional morphology as well as shape could be designed and fabricated.

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