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3-D Fabrication Technology for Tissue Engineering

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2.1. INTRODUCTION

Tissue engineering typically involves the combination of cells and biomaterials to form tissues with the goal of replacing or restoring physiological functions lost in diseased organs. The biomaterial scaffolds are designed to provide mechanical support for the cells; however, in practice, the simple addition of cells to porous scaffolds often does not recapitulate sufficient tissue function. Scaffold design previously focused on the incorporation of macroscale features such as interconnected pores for nutrient transport and tissue remodeling. One strategy to further augment the function of tissue-engineered constructs is to mimic the *in vivo* tissue microarchitecture and cellular microenvironment. Tissues in the body are divided into repeating functional units (e.g., nephron, islet) [1], whose 3-D architecture coordinates the processes of multiple types of specialized cells. Further, the local environment of these cells presents biochemical and physical stimuli that specifically modulate both cellular functions, e.g. biosynthesis and metabolism, and cellular fate processes such as differentiation, proliferation, apoptosis and migration. Thus, the fabrication of functional 3-D tissue constructs that incorporate both microscale features for appropriate cell functions and macroscale mechanical and transport properties demands control over chemistry and architecture over multiple length scales.

Tissue engineering scaffolds that mimic the complex architecture of native tissues have been more difficult to produce than conventional porous polymer scaffolds that support undirected cell adhesion and spreading within homogeneous and relatively large

(millimeter scale) constructs [2]. Recently, computer-controlled rapid-prototyping technologies have been adapted toward the fabrication of 3-D scaffolds with precise geometric control at the macro- and micro-scale. These 3-D fabrication approaches offer numerous opportunities with great potential for tissue engineering. For example, the function of complex tissue units is expected to rely on the independent control of macro- and micro-scale features. The incorporation of vascular beds would allow for larger constructs than could be supported by nutrient diffusion alone. In addition, the combination of clinical imaging data with CAD-based freeform techniques allows the fabrication of replacement tissues that are customized to the shape of a particular defect. Finally, the large-scale production of identical functional tissue units may find use in cell-based assays for drug discovery or for fundamental biological studies. Recently, microfabrication tools have been applied to the study of cell-cell and cell-matrix interactions within a two-dimensional cell culture context [1]. Extending these studies to three dimensional cellular control may provide further insight on cellular interactions and structure/function relationships within a tissue.

In this review, we describe various three-dimensional technologies used in tissue design and fabrication and compare their modes of assembly, spatial resolution, development stage, and feasibility for tissue engineering. Specifically, our discussion focuses on three general approaches (Fig. 2.1): acellular polymer scaffold fabrication, cellular assembly techniques, and hybrid cell/scaffold strategies.

2.2. FABRICATION OF ACELLULAR CONSTRUCTS

Traditional scaffold fabrication methods, including solvent-casting/particulate-leaching, gas foaming, fiber bonding, phase separation, and emulsion freeze drying, allow for limited control of pore size and shape but lack the sensitivity to precisely determine scaffold architecture [3]. In contrast, CAD-based rapid prototyping methods provide excellent spatial control over polymer architecture and have recently been applied to the fabrication of 3-D tissue engineering scaffolds. In Figure 2.1, various methods for creating acellular scaffolds are categorized according to their modes of fabrication, using heat, light, adhesives, or molding. These techniques are presented below, along with recent applications and advances.

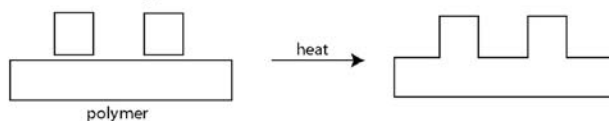
2.2.1. *Heat-Mediated 3D Fabrication*

Fabrication by heat energy combines pre-fabricated polymer layers into simple three-dimensional structures by raising the polymer above its glass transition temperature and fusing the softened layers together with applied pressure [4]. In sheet lamination fabrication, laser-cut polymer sheets are sequentially bonded by the application of heat and pressure. Currently, scaffolds created with this method have very low void volume and are generally too dense for the construction of tissues with high cellularity.

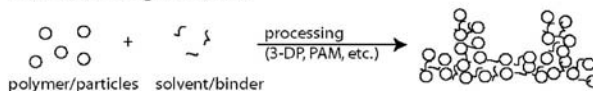
Lamination techniques can also be used to fabricate more intricate scaffolds that contain small, well-defined pores to increase void volume. For example, biodegradable polyester polymers such as poly(DL-lactic-co-glycolic) acid (PLGA), have been micropatterned by various techniques and laminated into three-dimensional structures. Borenstein and colleagues constructed thin biodegradable films containing small trenches by casting

Acellular Scaffold Fabrication

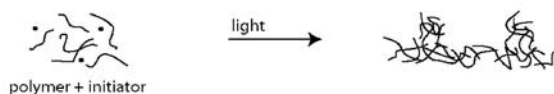
Fabrication using Heat



Fabrication using Adhesives



Fabrication using Light

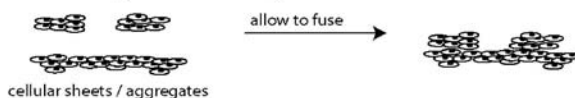


Fabrication by Molding



Cellular Constructs

Fabrication by Cellular Assembly



Fabrication of Hydrogel/Cell Hybrids

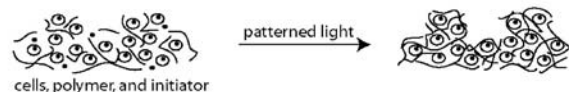


FIGURE 2.1. Summary of 3-D Scaffold Fabrication Methods. Acellular scaffolds can be fabricated using various techniques, such as heat (FDM), chemicals (3-DP), light (SLA), and molding. Cells themselves can be incorporated in the fabrication process by cellular addition or by photopatterning of hydrogels.

PLGA onto microfabricated silicon masters. When laminated together, these patterned films formed a vascular tissue engineering scaffold with 20 μm diameter channels between layers (Fig. 2.2a) [5]. Researchers later developed similar scaffolds with soft lithography techniques that utilize inexpensive elastomeric polydimethylsiloxane (PDMS) molds cast from silicon masters [6]. By introducing a PLGA solution into the mold and heating, Bhatia and colleagues created polymer layers that exhibited microstructures similar in shape and resolution (20–30 μm) to those on the silicon master and could be fused together (Fig. 2.2b) [7]. To further increase the scaffold surface area for cell attachment and proliferation,

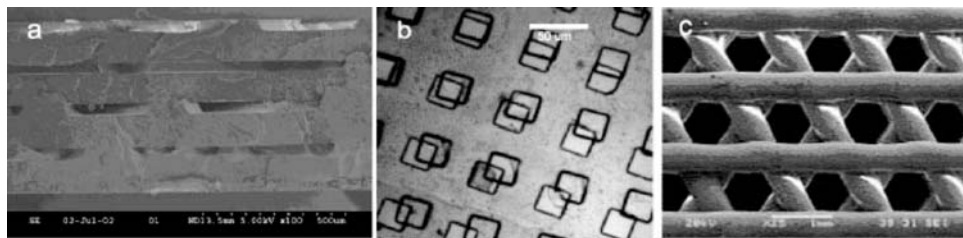


FIGURE 2.2. Fabrication using Heat. (a–b) Molded Lamination. Membranes of the biodegradable polymer PLGA are cast from silicon (a) or PDMS (b) molds and then laminated to create 3-D scaffolds. In (a), layers of PLGA are fused together to form microfluidic channels for vascular tissue engineering (c) Fused Deposition Modeling. Molten biomaterials are extruded through a nozzle to build 3-D scaffolds layer by layer. (Photo courtesy of Jeff Borenstein and Kevin King, Draper Laboratory).

micropores can be incorporated into the patterned PLGA membranes by solvent casting and particulate leaching strategies.

Selective laser sintering (SLS) is a heat-based fabrication technique that uses laser energy to combine powdered polymeric materials into defined shapes. A laser beam directed across a powder bed locally increases polymer temperature to fuse with the surrounding material and form a layer of patterned structures [4]. Three-dimensional SLS scaffolds are created sequentially with fresh powder deposited over each patterned layer. Unfused powder released from the scaffold yields high porosity and surface area while retaining mechanical integrity. The pattern resolution of SLS is limited by the diameter of the laser beam diameter to about 400 μm [4], and maximum pore size is about 50 μm due to the powder particle size [8]. Lee and Barlow first utilized SLS with polymer-coated calcium phosphate powders to fabricate oral implants and demonstrated extensive bone tissue ingrowth in dog models [9]. Since then, Leong and others have broadened SLS utilization for various biopolymer applications [8].

Fused deposition modeling (FDM) combines heat and extrusion techniques to create 3-D scaffolds layer by layer. A nozzle directs a stream of molten plastic or ceramic onto a previously deposited layer of material. By altering the direction of material deposition with each layer, scaffolds with complex internal organization can be formed (Fig. 2.2c). Zein and Hutmacher used this method to produce biodegradable poly(ϵ -caprolactone) (PCL) scaffolds exhibiting various honeycomb geometries with finely tuned pore and channel dimensions of 250–700 μm [10]. Primary human fibroblasts cultured in these scaffolds proliferated and produced extracellular matrix [11], and scaffolds composed of other bio-compatible polymers and composites have demonstrated utility for various tissue engineering applications [12–14]. While FDM exhibits high pattern resolution in the xy-plane, it is limited in the z-direction by the diameter of the extruded polymer filament that defines layer thickness and corresponding pore height. Further, high processing temperatures limit the biomaterials that are compatible with the method. However, FDM capabilities are expanding with new developments such as multi-phase jet solidification (MJS), a technique that allows simultaneous extrusion of multiple melted materials [15].

3-D plotting is a similar heat-based extrusion technology that is not limited to synthetic polymers that must withstand high temperatures while retaining their desired properties such as degradation and biocompatibility. Instead, fabrication is based on a sol-gel phase

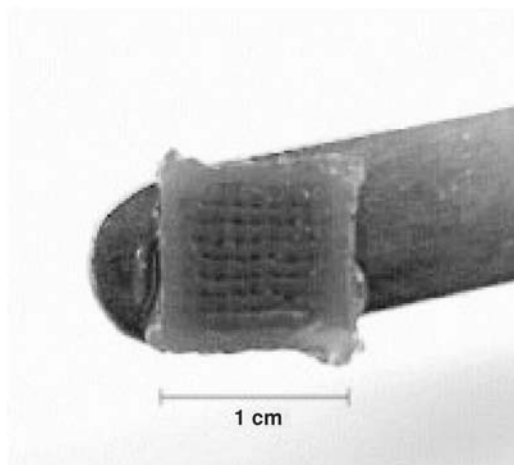


FIGURE 2.3. 3-D Plotting. Heated liquid agar solidifies into a 3-D hydrogel scaffold when deposited into a cooled medium. (from [16], reprinted with permission of Elsevier).

transition that occurs at lower temperatures. This strategy has been demonstrated with natural hydrogel biomaterials that are substantially more versatile for tissue engineering applications. For example, Mulhaupt and coworkers deposited agar and gelatin solutions heated to 90°C into a cooled plotting medium, resulting in a 3-D hydrogel scaffold (Figure 2.3) [16]. Similarly, Ang and colleagues used robotic dispensing to form chitosan and chitosan-hydroxyapatite scaffolds [17]. Following fibrin treatment, these scaffolds supported the adhesion of human osteosarcoma cells or mouse fibroblasts.

2.2.2. *Light-Mediated Fabrication*

Light energy can also be used to fabricate structured 3-D polymer scaffolds. Photopolymerization uses light to initiate a chain reaction that solidifies a liquid polymer solution. Stereolithography (SLA) is a photopolymerization method that utilizes a deflected UV laser beam to irradiate and solidify exposed polymer regions at the surface of a vat of photo-sensitive polymer (Fig. 2.4). Multiple layers are formed sequentially by lowering the stage and repeating the laser illumination. While SLA machines are traditionally used to build prototypes and molds for implants, Cooke et al. fabricated biodegradable 3-D polymer scaffolds for bony tissue consisting of diethyl fumarate, poly(propylene fumarate) and the photoinitiator bisacylphosphine oxide [18]. Similarly, a photocurable ceramic acrylate suspension formed cancellous bone [19] and hydroxyapatite bone tissue scaffolds [20], with overall dimensions suitable for healing critical-sized (4-mm thickness, 50-mm diameter) bone defects. As with SLS, stereolithography is limited in resolution by laser beam diameter to approximately 250 μm , although small-spot laser systems have demonstrated the production of smaller (70 μm) features [4].

Light energy can also be used to photopolymerize hydrogel polymer scaffolds that are less rigid than conventional stereolithography materials. Hydrogels are crosslinked networks of insoluble hydrophilic polymers that swell with water. Their increasing popularity as tissue

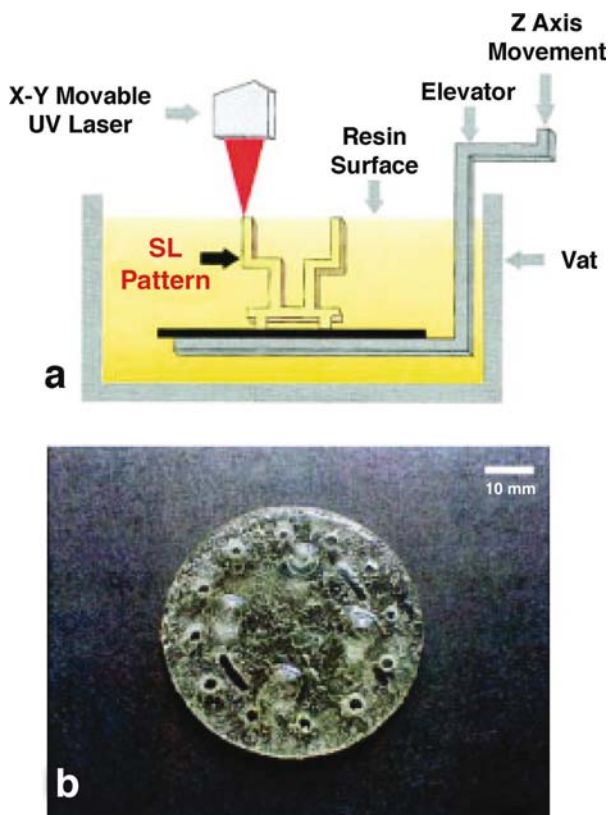


FIGURE 2.4. Stereolithography. (a) UV light is used to crosslink the material in specific regions of a layer. The elevator is then lowered to reveal a new layer of polymer, and the process is repeated to create the desired shape. (b) A prototype scaffold designed using SLA (from [18], reprinted by permission of John Wiley & Sons, Inc.).

engineering biomaterials reflects mechanical properties and high water content analogous to those of natural tissue. Yu and colleagues demonstrated a photolithographic method of patterning layers of dried 2-hydroxyethyl methacrylate that were subsequently rehydrated and seeded with cells [21]. However, the resolution of hydrogel scaffold fabrication may be compromised during rehydration of the polymer. Instead, Matsuda et al. later created scaffolds with improved strength and limited swelling using combinations of vinylated polysaccharides and diacrylated polyethylene glycol [22]. Additionally, the photopatterning of hydrogels has recently been extended to incorporate living cells into hybrid constructs, as discussed in a later section.

2.2.3. Adhesive-Mediated Fabrication

Scaffolds fabricated by binding polymers with solvents or adhesives, rather than by heat or light, circumvent biomaterial limitations for thermostable polymers or for biocompatible photoinitiators. Three-dimensional printing (3-DP), for instance, utilizes an ink jet printer to deposit a binder solution onto a polymer powder bed. Multiple layers can be fabricated and stacked with dimensions on the scale of polymer particle size (approximately 200–300 μm)

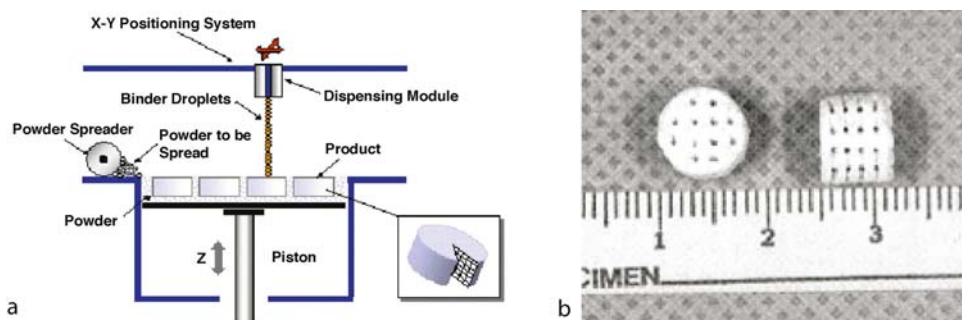


FIGURE 2.5. 3-D printing. Ink jet technology is used to print a binder solution onto a bed of polymer powder. An additional layer of powder is then deposited, and the process is repeated to form 3-D scaffolds (a) from Therics, website, with permission; (b) from [24], reprinted with permission of Leppincott Williams & Wilkins.

(Fig. 2.5) [23]. Scaffolds composed of natural biopolymers such as starch, dextran, and gelatin can be formed using aqueous solvents, and further can incorporate micropores by particle leaching. Griffith et al. explored porous scaffolds of PLGA for liver tissue engineering and demonstrated rat hepatocyte attachment [24]. Others have extended this technique to examine the effects of pore size on the attachment, growth, and matrix deposition of different cell types [25].

Pressure assisted microsyringe (PAM) fabrication is another adhesion-based technique that uses a solvent to bind polymers in a layer by layer format. A stage controlled microsyringe delivery system deposits a stream of polymer dissolved in solvent through a 10–20 μm glass capillary needle [7]. The polymer stream thickness can be modified by varying the solution viscosity, syringe-tip diameter, syringe pressure, and stage motor speed, to generate structures that range in dimension from 5 μm to 600 μm . This method is similar to FDM scaffold fabrication, but is capable of high resolution features and does not require heat. However, the limited size of the syringe-needle system prohibits the use of particulate leaching to increase microporosity and scaffold surface area.

2.2.4. Indirect Fabrication by Molding

In addition to the methods described above that directly fabricate 3-D scaffolds, scaffolds can also be cast from microstructured molds formed using the same methods. This indirect fabrication strategy is advantageous for sensitive biomaterials that are incompatible with fabrication conditions, since only the mold itself is subjected to the processing environment. Further, the resulting scaffold represents an inverse of the mold, thereby extending the 3-D design possibilities. For example, Orton et al. casted a hydroxyapatite/acrylate suspension onto a negative epoxy mandible mold made by stereolithography (Fig. 2.6a) [26]. After heat-curing the polymer, the mold and acrylate binder were incinerated. The resulting hydroxyapatite scaffolds contained different internal channel architectures and resulted in bone ingrowth in minipigs up to nine weeks post-implantation [27]. Others have created molds for indirect scaffolds using 3-DP by depositing wax or other low melting point compounds that can be later removed with elevated temperature or solvents. This method has been combined with particulate leaching to indirectly fabricate porous scaffolds composed of hydroxyapatite, poly(L)lactide, and polyglycolide [28]. Sachlos et al. similarly used ink

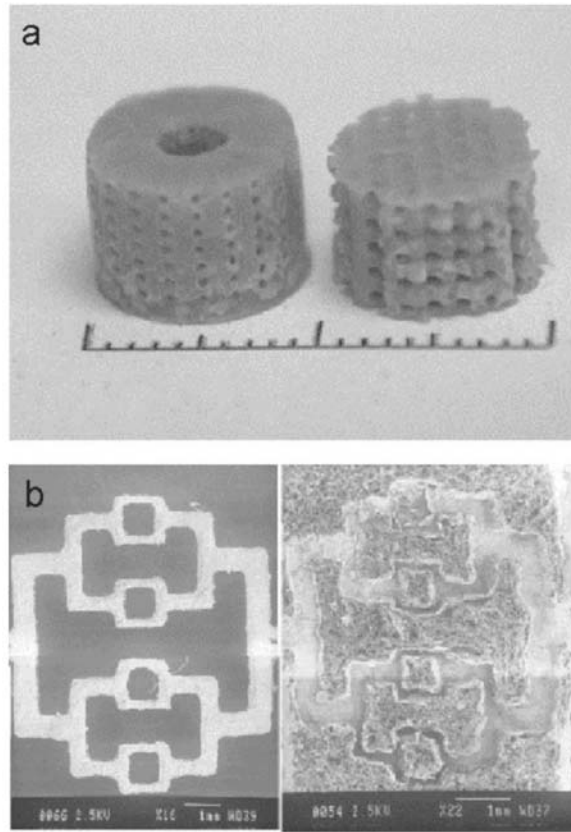


FIGURE 2.6. Molded Scaffolds. (a) Hydroxyapatite was cast into a negative epoxy mold (manufactured using stereolithography) and then cured by heat. The scaffold was then placed in a furnace to burn out the mold. (b) The extracellular matrix compound collagen was cast onto a negative mold that was printed using ink jet technology. The mold was then dissolved away with ethanol, leaving a patterned collagen scaffold (from [29], reprinted with permission of Elsevier).

jet printing to manufacture molds for casting collagen microstructures with 200 μm feature size (Fig. 2.6b) [29]. The molds were then dissolved with ethanol to form scaffolds with predefined internal morphology. Furthermore, these scaffolds would present a component of extracellular matrix specifically recognized by cells to enhance attachment. However, implantation of such a construct may also be problematic in that other host cells may also react and respond in a nonspecific manner.

2.3. FABRICATION OF CELLULAR CONSTRUCTS

Although acellular assembly techniques have proven useful for defining the macroscopic and microscopic features of a 3-D scaffold, these methods are generally limited by

inefficient and inhomogenous incorporation of cells. The direct assembly of cultured layers of living cells is an alternative being pursued by several groups. This strategy was demonstrated for myocardial tissue by culturing cardiomyocytes on dishes selectively grafted with poly(N-iso-propylacrylamide) (PIPAAm) to form a substrate with temperature-sensitive adhesive properties. At a reduced temperature, the grafted polymer hydrated and promoted the release of a cellular sheet that retained cell-cell junctions and extracellular matrix and could be layered with additional sheets [30]. In a similar manner, Okano and colleagues created corneal epithelial sheets from corneal stem cells without the use of polymer scaffolds, and transplanted sheets remained stable for up to six months in rabbit models [31]. Auger and colleagues have also adapted this technique to vascular tissue engineering. To mimic the structure of blood vessels, they wrapped smooth muscle cell sheets around a tubular mandrel and subsequently seeded endothelial cells within the lumen of the cylinder [32]. After culture with pulsatile flow, the multilayer engineered blood vessels demonstrated excellent mechanical properties and exhibited cellular markers that resembled native vessels.

These cell layering techniques have limited capability for the formation of complex 3-D patterned structures. Instead of using cell monolayers that fuse into a sheet, some groups have achieved greater complexity of tissue construction by the selective delivery and spontaneous fusion of living cells into 3-D structures. For example, Mironov et al. used a jet-based printer to position cell aggregates and embryonic heart mesenchymal fragments that fused together within biocompatible gels of varying chemical and mechanical properties [33]. In the future, this ‘organ-printing’ technology may allow for precise 3-D cell positioning that could be scaled up to larger tissue engineered constructs [34, 35]. Odde et al. have explored the use of laser-based optical forces to precisely deliver a stream of living cells and ‘write’ them into arbitrary positions on a substrate [36]. While single cells can be positioned in this manner, the serial deposition process may pose problems for scaling up to tissue dimensions. Further characterization of these constructs will be required to determine which tissues are amenable to fabrication by these emerging cellular assembly techniques.

2.4. FABRICATION OF HYBRID CELL/SCAFFOLD CONSTRUCTS

One disadvantage of direct cellular assembly is that constructs may not possess adequate mechanical stability for tissue engineering applications. Conversely, acellular scaffolds have excellent mechanical strength but may be difficult to populate with cells. Hydrogel polymers have the ability to provide both structural support and high tissue density while maintaining an *in vivo*-like environment for cells [37]. Many of these water-swollen polymers can be formed in mild conditions compatible with living cells, allowing the formation of hybrid cell/scaffold constructs. A significant advantage of this strategy is that the construct can then encapsulate a homogeneous cell population. The construct shape is determined either by the mold or container used during crosslinking, or by spatial photopatterning using selective light exposure.

2.4.1. Cell-laden Hydrogel Scaffolds by Molding

Hybrid cell/hydrogel constructs conform to the dimensions of the mold or container used. Initial studies generated constructs containing a random distribution of cells in the

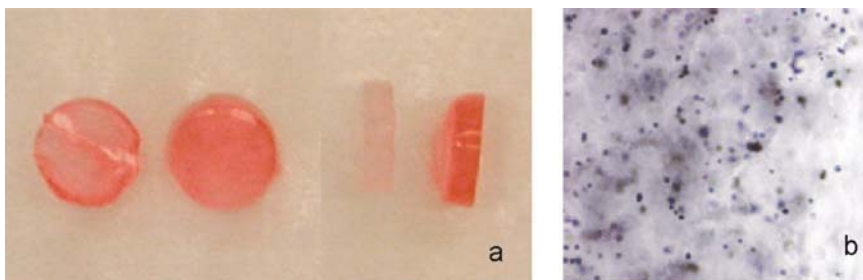


FIGURE 2.7. PEG-based hydrogels containing cells. (a) PEG-based hydrogels are crosslinked to form the shape of the container (dye added for clarity). (b) Living cells are suspended within the crosslinked hydrogel (MTT stain for viability). (Photos courtesy Jennifer Elisseeff, Johns Hopkins University)

shape of thin sheets or disks by polymerization within tissue culture wells or tubes (Fig. 2.7). In this manner, Hubbell and coworkers explored the encapsulation of cells in various biological hydrogels, such as collagen and fibrin, that were functionalized with genetically engineered bioactive sites to enhance cell adhesion and proteolytic remodeling [38]. Desai et al. extended these studies by using microfluidic molding technologies to deposit micropatterned collagen gel structures containing living cells [39]. While complex biological microstructures containing a few layers were fabricated in this manner, microfluidic molding is typically constrained to flat surfaces and may be difficult to generalize to 3-D tissue architectures.

Synthetic polymer hydrogels offer several advantages over biological hydrogels for producing scaffolds containing homogeneously dispersed cells. In particular, poly(ethylene glycol) (PEG)-based hydrogels are increasingly used for cell encapsulation because of their biocompatibility, hydrophilicity, and customizable mechanical and transport properties by changing the monomer chain length and polymer fraction. Photopolymerizable PEG hydrogels have been shown in numerous studies to support the viability and function of various immobilized cell types, including osteoblasts [40], chondrocytes [41, 42], vascular smooth muscle cells [43], and fibroblasts [44]. Additionally, these hydrogels can be chemically functionalized by incorporating biologically relevant molecules [45]. For example, extracellular matrix protein domains were shown to enhance cell attachment and incorporation [43, 46–49], tethered growth factors modulated cell functions [46], and degradable/cleavable linkages within the polymer backbone allowed cell proliferation and migration [43, 44, 46, 47, 50–53]. Thus, photopolymerized synthetic hydrogels are particularly appealing for tissue engineering because of their controllable biochemical and mechanical properties, gentle crosslinking processes that are compatible with living cells, and hydrated, tissue-like 3-D environment.

2.4.2. Cell-laden Hydrogel Scaffolds by Photopatterning

Although molding techniques can form hybrid cell/hydrogel constructs with micropatterned external features, they are not amenable to patterning internal structure of complex engineered tissues. However, recent developments have exploited the ability to localize light exposure, and therefore hydrogel crosslinking, in defined micropatterns, potentially

allowing the buildup of complex microstructures in a layer by layer manner. Outside the tissue engineering arena, hydrogel microstructures have been formed by photolithographic patterning with applications such as microfluidic valves [54] and cell-laden microstructures on silicon [55].

Liu and Bhatia recently adapted photolithographic techniques to existing PEG-based cell-encapsulation chemistry and created organized, three-dimensional cell/hydrogel networks (Fig. 2.8) [56]. In this method, living cells were suspended in a polymer solution and exposed to UV light through a photolithographic mask to form multiple cellular domains

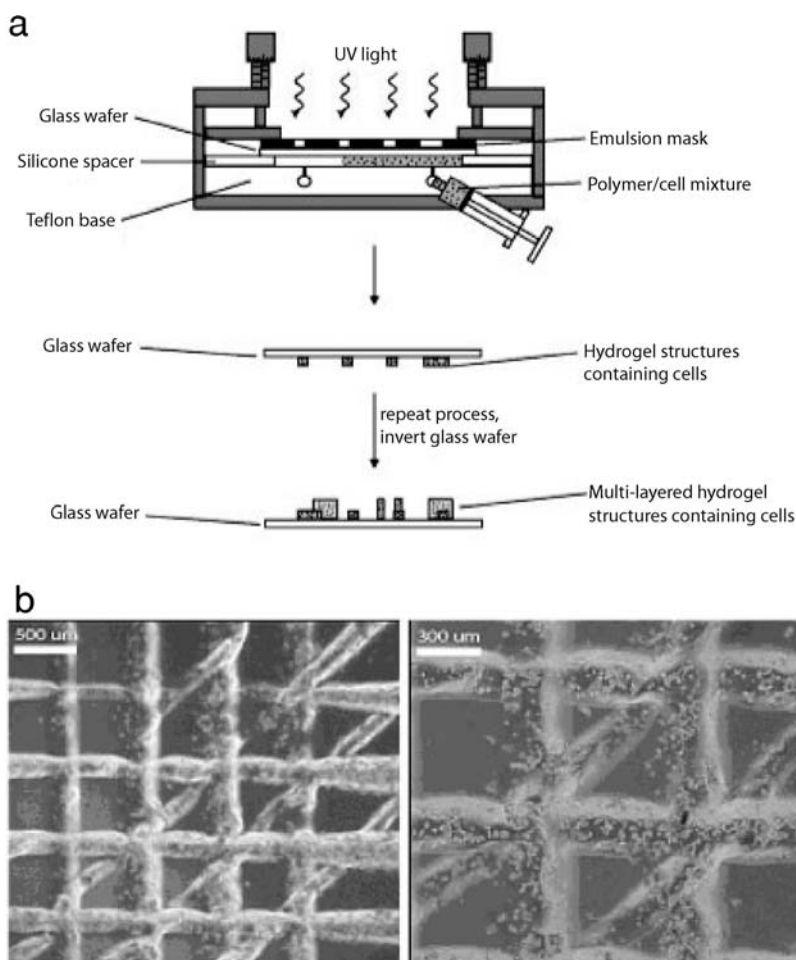


FIGURE 2.8. 3-D Photopatterning of Hydrogels. (a) Photopatterning Method. Polymer solution and cells are introduced into a chamber. The unit is exposed to 365 nm light through an emulsion mask, causing crosslinking of the polymer in the exposed areas and trapping the cells within these regions. The uncrosslinked polymer solution and cells are then washed away, and the process is repeated with thicker spacers and a new mask to create 3-D cellular hydrogel structures. Each layer may contain the same type of polymer/cell mixture, or can be composed of different polymer properties or different cell types. (b) Three layered hydrogel structure containing cells (from [56]).

with controlled hydrogel architecture. Uncrosslinked polymer and cells were then rinsed away and additional domains could be photopatterned with different cell types, polymer formulations, and exposure patterns. During each exposure cycle, the newly crosslinked polymer fused with existing hydrogel domains. Furthermore, 3-D multilayer constructs with complex internal structure were formed by increasing the height of the photocrosslinking chamber between exposure steps. In this manner, a three layered hydrogel construct was fabricated with raised protrusions containing a high cell density (Fig. 2.8b). To date, microstructure feature size has approached 50 μm , thus enabling the patterning of cells on the scale of functional tissue units. While these structures contain randomly dispersed cells, Albrecht et al. have developed a complimentary technology capable of defining the organization of encapsulated cells within a hydrogel to a resolution of $<10\text{ }\mu\text{m}$ [57, 58]. This method utilizes electromagnetic fields to specify the position of cells in the liquid polymer solution prior to photocrosslinking. In conjunction with bioactive hydrogel technologies being explored by numerous groups, the photopatterning of hydrogels containing homogeneous or organized patterns of living cells may lead to the development of improved tissue engineered constructs with customized spatial, physical, and chemical properties. The flexibility of these hydrogel systems shows great promise for the fabrication of 3-D tissues that mimic the structural, multicellular, and biochemical complexity found in many organs in the body.

2.5. FUTURE DIRECTIONS

Novel scaffold fabrication methods, often based on technologies borrowed from the manufacturing industry, have led to rapid progress in the development of complex 3-D tissue engineered constructs in recent years. The various acellular, cellular, or hybrid cell/scaffold fabrication strategies described in this review are summarized in Table 2.1 with regard to their spatial resolution, advantages, and limitations. The utility of each fabrication method will ultimately depend on design criteria specific to each tissue engineering application, including chemical composition, mechanical strength, degradation profile, nutrient transport, and cellular organization.

The field of tissue engineering has advanced significantly from the initial examples of seeding living cells into synthetic polymer scaffolds to the development of tissue constructs with physical and biochemical complexity. As researchers develop a greater understanding of the biology underlying fundamental structure-function relationships, factors that influence cell fate (e.g., growth, morphogenesis, apoptosis) and function (gene expression, biosynthesis) can be incorporated into the design of tissue engineering strategies. These factors include signals from the cellular microenvironment that are sensed in a three-dimensional context, such as cell-cell and cell-matrix interactions, soluble signals, and mechanical forces. The ability to control the presentation of microenvironmental cues at the microscale (cell and functional subunit, 10–100 μm) as well as bulk properties at the macroscale (tissue implant, 1–100 mm) will be enabled by leveraging these emerging 3-D fabrication technologies. While the goal of engineering complex tissues remains a difficult challenge, continued interaction between interdisciplinary fields of cell and molecular biology, chemistry, biomaterials, and medicine, will ensure continued progress toward substantial improvements in human health.

TABLE 2.1. Comparison of 3-D scaffolding methods.

	Resolution (μm)	Advantages	Disadvantages
ACELLULAR 3-D SCAFFOLDS			
		use of well-established fabrication methods, usually automated	must seed cells post-processing, less control in cell placement and distribution
<i>Heat-Mediated Fabrication</i>			
Micro Molding [5, 7]	20–30	simple; reusable molds	limited to thin membranes, each layer must be contiguous structure, manual alignment required
Selective Laser Sintering [4, 8, 9]	400	high porosity, automated	high temperatures during process, powder may be trapped
Fused Deposition Modeling [8, 10, 11]	250–700	no trapped particles or solvents, automated	high temperatures during processing
3-D Plotting [16]	1000	use of hydrogel materials (agar, gelatin), automated	limited resolution
<i>Light-Mediated Fabrication</i>			
Stereolithography [4, 18]	70–250	ease of use, easy to achieve small features, automated	limited choice of materials- must be photosensitive and biocompatible; exposure of material to laser
<i>Adhesive-Mediated Fabrication</i>			
3-D Printing [23–25]	200–500	versatile; high porosity, automated	limited choice of materials (e.g. organic solvents as binders); difficult to reduce resolution below polymer particle size
Pressure Assisted Microsyringe [7]	10	high resolution, not subject to heat, automated	viscosity dependent, no inclusion of particles
<i>Indirect Fabrication by Molding</i>			
Matrix Molding [29]	200	use of biological matrix materials (collagen), mold fabrication can use automated methods (above)	features must be interconnected, weaker mechanical properties
CELLULAR 3-D SCAFFOLDS			
		Precise placement of cells throughout construct, ability to place multiple cell types arbitrarily	limited fabrication conditions (sterility, temperature, pH), still in earlier phases of development
<i>Cellular Assembly</i>			
Organ Printing [33, 35]	100	incorporation of cell aggregates or tissue explants, precise cell placement, automated	lack of structural support, dependence on self assembly
Laser-Guided Deposition [36]	<1	precise single cell placement, automated	has yet to be extended to 3-D structures, lack of structural support

(cont.)

TABLE 2.1. *Continued*

	Resolution (μm)	Advantages	Disadvantages
Hybrid Cell/Scaffold Assembly			
Hydrogel Photopatterning [56]	100	incorporation of living cells within scaffold, leverages existing hydrogel chemistry (incorporation of peptides, degradation domains), versatile	not yet automated, exposure of cells to ultraviolet light, diffusion of large molecules limited by hydrogel pore size

ACKNOWLEDGEMENTS

We would like to thank the Whitaker Foundation (V.L.T., D.A.), American Association of University Women (V.L.T.), NIH NIDDK, NSF CAREER, David and Lucile Packard Foundation, and NASA for their generous support.

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BioMEMS and Biomedical Nanotechnology

Volume III: Therapeutic Micro/Nanotechnology

Editor-in-chief: Ferrari, M. - Desai, T.; Bhatia, S.N. (Eds.)

2007, XXIV, 376 p., Hardcover

ISBN: 978-0-387-25565-1