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Fabrication of PLGA scaffolds using soft lithography and microsyringe deposition

Giovanni Vozzi[a], Christopher Flaim[b], Arti Ahluwalia[a,1], Sangeeta Bhatia[b,*,1]

[a] *Centro Interdipartimentale di Ricerca "E. Piaggio", Faculty of Engineering, University of Pisa-Via Diotisalvi, 2-56100 Pisa, Italy*
[b] *Departments of Bioengineering and Medicine, University of California-San Diego, 9500 Gilman Drive, MC 0412, La Jolla, CA 92096, USA*

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Abstract

Construction of biodegradable, three-dimensional scaffolds for tissue engineering has been previously described using a variety of molding and rapid prototyping techniques. In this study, we report and compare two methods for fabricating poly(DL-lactide-*co*-glycolide) (PLGA) scaffolds with feature sizes of approximately 10–30 μm. The first technique, the pressure assisted microsyringe, is based on the use of a microsyringe that utilizes a computer-controlled, three-axis micropositioner, which allows the control of motor speeds and position. A PLGA solution is deposited from the needle of a syringe by the application of a constant pressure of 20–300 mm Hg, resulting in a controlled polymer deposition. The second technique is based on 'soft lithographic' approaches that utilize a poly(dimethylsiloxane) mold. Three variations of the second technique are presented: polymer casting, microfluidic perfusion, and spin coating. Polymer concentration, solvent composition, and mold dimensions influenced the resulting scaffolds as evaluated by light and electron microscopy. As a proof-of-concept for scaffold utility in tissue engineering applications, multilayer structures were formed by thermal lamination, and scaffolds were rendered porous by particulate leaching. These simple methods for forming PLGA scaffolds with microscale features may serve as useful tools to explore structure/function relationships in tissue engineering.

Keywords: Microfabrication; PLGA; Microsyringe; Soft lithography

1. Introduction

The fabrication of three-dimensional (3D) scaffolds that mimic the in vivo cellular microenvironment is of fundamental importance to the success of tissue engineered constructs. Both scaffold chemistry and architecture can influence the fate and function of engrafted cells [1,25,28]. With regard to architecture, macroscopic 3D shapes are typically defined by traditional processes such as extrusion, melt molding, and solvent casting [2–6]. Material microstructure, in contrast, is often controlled by process parameters such as the choice of solvent in phase separation, doping with particulate leachants, gas foaming, woven fibers, and controlled ice crystal formation and subsequent freeze-drying to create pores [7–13]; however, these scaffolds lack a well-defined organization that is found in most tissues in vivo.

At the microscale, techniques to control the architecture of biodegradable polyester scaffolds, such as poly(DL-lactide-*co*-glycolide) (PLGA), are being developed and described in the literature. For example, a Fused Deposition Modelling (FDM) method can create solid objects with ~250 μm resolution using a robotically controlled miniature extruder head [14]. Biodegradable polymer membranes of thickness between 500 and 2000 μm cut by laser can be laminated to produce structures with 100 μm resolution [15]. By exploiting computer-aided design and solid free form fabrication, both 3D-printing and lost mold methods have been developed. 3D-Printing employs polyester particles that are bound together by the application of chloroform from an inkjet head with a resolution of approximately 300 μm [16,17]. Similarly, the lost mold technique uses

*Corresponding author. Tel.: +1-858-822-3142; fax: +1-858-822-4203.
E-mail addresses: arti.ahluwalia@ing.unipi.it (A. Ahluwalia), sbhatia@be-research.ucsd.edu (S. Bhatia).
[1] S.N.B. and A.A. share senior authorship on this work.

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stereolithography to fabricate an epoxy mold that is lost when the surrounding ceramic is heated, with a resolution of approximately 450 μm [18]. Although complex objects can be created using these various technologies, the ability to reproducibly and simply fabricate polymer scaffolds with tissue-scale features (i.e. 10–100 μm) for the investigation of fundamental structure/function relationships has not been reported.

In this paper, we present two new fabrication methods for biopolymer scaffolds using PLGA as a prototypic polymer for tissue engineering applications. The first technique, pressure assisted microsyringe (PAM), is an automated system using a microsyringe and stage controller. The second method is an adaptation of so-called 'soft lithography' that utilizes elastomeric polydimethylsiloxane (PDMS) replicas of microfabricated masters [19–24]. The latter method also allows for the introduction of porosity in the structure. Scaffolds fabricated using both techniques were used to form multilayer structures by membrane lamination. Finally, we compare the advantages and limitations of each technique with regard to resolution, cost, and process variables.

2. Materials and methods

2.1. Polymer solution

The PLGA solution was obtained by dissolving 85/15 PLGA (Birmingham Polymers Inc., Birmingham, AL, USA, MW 18,000) in chloroform to give the desired concentration. This solution was used for all fabrication techniques, except where noted.

2.2. Pressure assisted microsyringe

The first fabrication technique, developed at the Interdepartmental Research Center "E: Piaggio" at the University of Pisa, is based on the use of a microsyringe that allowed the deposition of a wide range of polymers [26]. PAM-based microfabrication was used to fabricate 2D and 3D scaffolds of biodegradable polymers. The system, illustrated in Fig. 1, consists of a stainless-steel syringe with a 10–20 μm glass capillary needle. A solution of the polymer in a volatile solvent is placed inside the syringe and expelled from the tip by the application of filtered compressed air. The syringe is mounted on the z-axis of a three-axis micropositioning system which was designed and built in-house and has a resolution of 0.1 μm. A supporting substrate, usually glass, is placed on the two horizontal motors and is moved relative to the syringe. The lateral dimensions of the structures deposited is between 5 and 600 μm, depending on the pressure applied to the syringe, the viscosity of the solution, the motor speed, and the

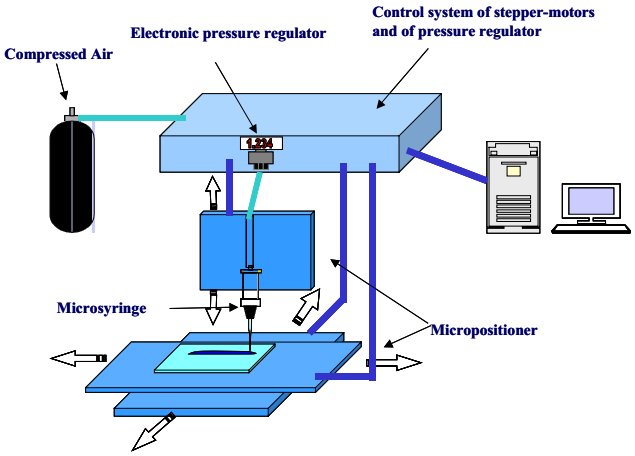


Fig. 1. Schematic of microsyringe method.

dimensions of the syringe tip. The system has been characterized, optimized, and a simple model simulating the fluid-dynamics of the deposition process has been developed [26]. The entire system including valves, pressure regulators, sensors and position controllers, is interfaced to and controlled by an IRIS card (Eclypse, Pisa, Italy) which enables the control of motor speeds (up to 10 cm/s in both x and y directions) and position. The control software was developed in C with a user-friendly graphic interface that allows a wide range of patterns with a well-defined geometry to be designed and deposited. After the first layer has been deposited, subsequent layers are deposited by moving the syringe up along the z-axis by an amount corresponding to the height of each layer. In theory, each layer can consist of a different polymer or pattern, thus allowing a wide range of 3D structures to be fabricated.

2.3. Soft lithographic techniques

Three variations of a technique for creating PLGA membranes from a PDMS mold were developed at the Microscale Tissue Engineering Laboratory, University of California, San Diego. The PDMS mold was cast from a microfabricated silicon master using methods previously described [32]. Briefly, silicon 100 wafers were spin-coated with EPON-SU8 photoresist (Microchem Co., Newton, MA), baked to drive away the solvent, and then exposed to UV light in a Bottom Side Mask Aligner (Karl Suss, Waterbury Center, VT) through a mask. The mask was created using Coreldraw 9.0 and printed on a transparency using a commercial Linotronic-Hercules 3300 dpi high-resolution line printer. Exposed photoresist was then developed (SU8 developer, Microchem Co.) and subsequently the wafers were baked. PDMS prepolymer was prepared by mixing the commercially available prepolymer and catalyzer (Sylgard 184 kit, Dow Corning) in a 10:1 w/w ratio. The

mixture was degassed under vacuum to eliminate bubbles created during mixing. The prepolymer solution was cast on the master and placed under vacuum once again to remove any bubbles that may have been introduced. PDMS was cured by baking for 2 h at 65°C. After cooling to room temperature, the PDMS was peeled from the silicon master. The mold was then washed with 70% ethanol and sonicated for 5 min prior to use.

2.3.1. Micromolding method

In this method, the PLGA solution was deposited on the PDMS mold and placed under vacuum for 2 min. During this time the polymer filled the microchannels present in the mold and displaced any air present. Once the polymer had filled the mold, excess PLGA was removed by dragging the edge of a glass slide across the top of the mold. The filled mold was baked for 30 min at 60°C. When cooled, the PLGA pattern was easily removed with a pair of tweezers. The steps in this method are summarized in Fig. 2a.

2.3.2. Microfluidic method

The second technique relied on the microfluidic channels that were created when a PDMS mold was sealed against a flat substrate. In our case, the PLGA solution was forced to flow through the channels by applying a negative pressure using a vacuum pump. When the polymer had completely filled the mold, the whole assembly (mold, polymer and support substrate) was baked for 30 min at 40°C. When cooled, the mold was peeled from the substrate leaving a thin membrane firmly attached to the substrate. The steps involved in this microfabrication method are summarized in Fig. 2b.

2.3.3. Spin coating

In the third variation of the method, a PLGA solution was spin-coated using a photoresist spinner at 2000 rpm

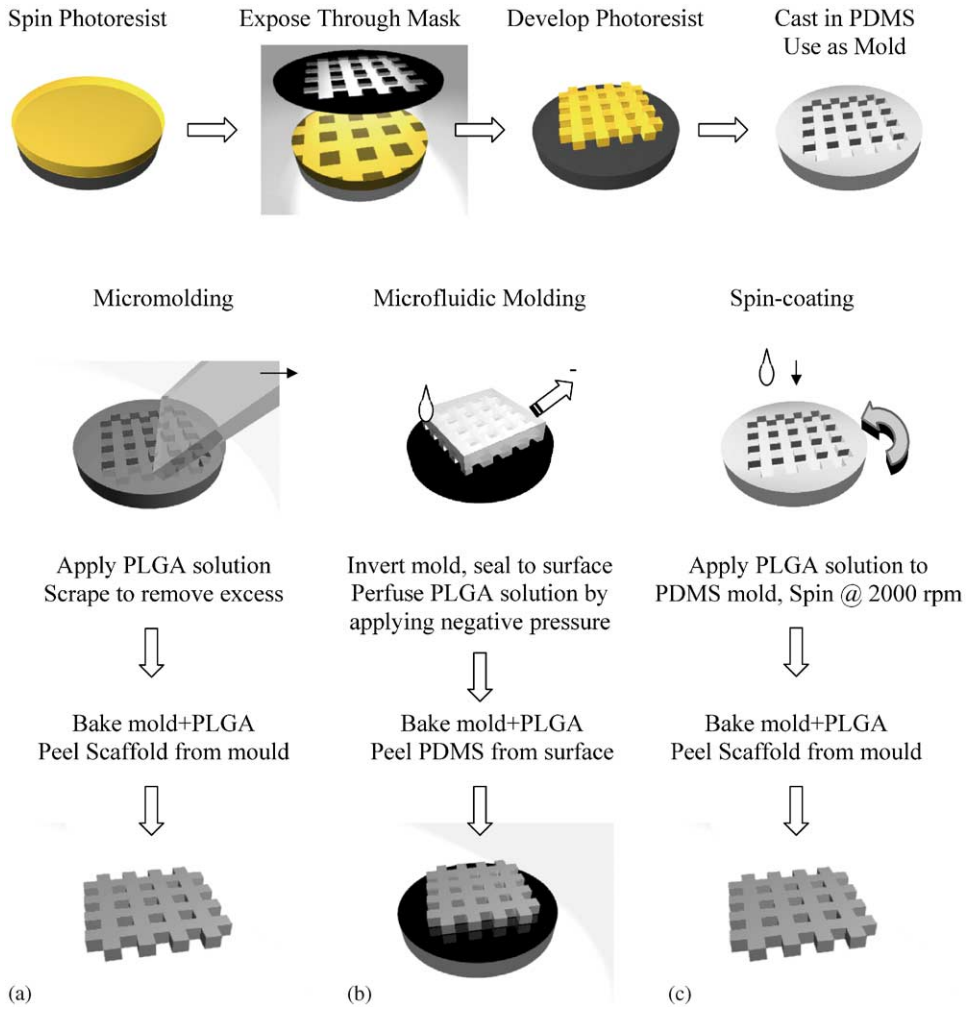


Fig. 2. Schematic of soft lithography methods. Photolithography is used to generate a polydimethylsulfoxide (PDMS) mold. (a) PDMS mold is used for solvent casting of PLGA. (b) PDMS mold is sealed against a solid surface to form a network of microfluidic channels. PLGA solution is perfused through the network and cured in place. (c) PDMS mold is spin-coated with PLGA solution to rapidly drive off the solvent and produce a scaffold.

onto a PDMS mold with ~30 μm feature heights. The mold was kept at room temperature for 1 h to allow for complete evaporation of the solvent. The scaffolds were then removed with tweezers. The steps involved in this method are summarized in Fig. 2c.

2.4. Application to tissue engineering

2.4.1. Multilayer lamination

Once several PLGA membranes had been fabricated, it was possible to construct multilayer structures by laminating them together. This was accomplished by clamping the edges of the patterns and heating for 10 min at 40°C.

2.4.2. Introduction of porosity by particulate leaching

In conjunction with the membrane fabrication techniques described above, it is also possible to create scaffolds with an internal microstructure. This was accomplished by mixing 20–65 μm glucose grains, which had been previously minced and sieved, with the polymer solution in a 1:1 weight ratio. To ensure that the polymer solution with glucose completely filled the mold, it was important that the solution not be too viscous. The optimum concentration of the polymer solution for this application was found to be around 5–10%. After the membrane was fabricated, the glucose particulates were removed by leaching in deionized water overnight.

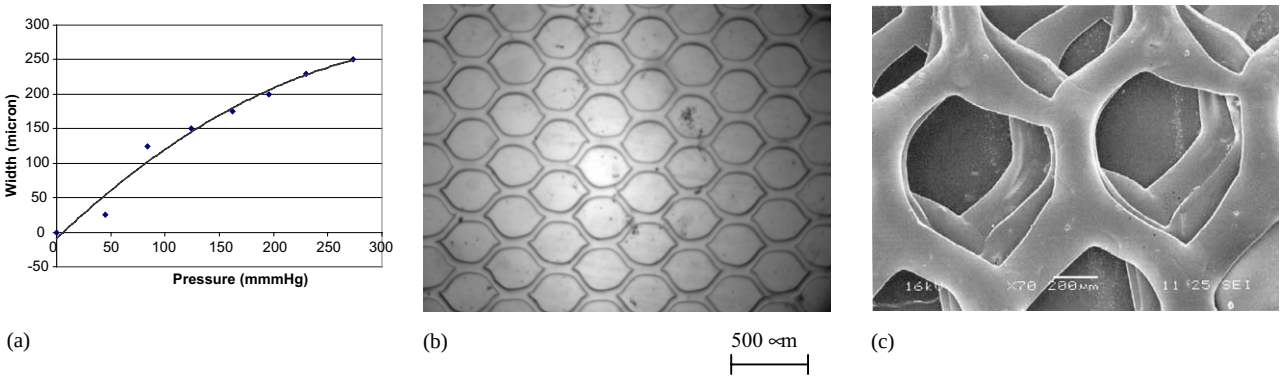
3. Results and discussion

We have presented and characterized two new microfabrication techniques that address the need for creating biopolymer scaffolds with organized microscale architecture for tissue engineering applications. The first is based on a PAM, and the second is based on soft lithography, of which three variations are described. Regardless of the technique used, the possibility of generating scaffolds with a well-defined geometry at the micron scale enables the study of the influence of topology on cellular activity, and can lead to the development of methods of engineering complex tissues.

3.1. Microsyringe

We sought to characterize the fluidic polymer deposition of the microsyringe system. Our model predictions [26] indicate that the width of the pattern can be controlled by a number of factors which include the diameter of the tip, the viscosity of the polymer solution, the applied pressure, and the motor speed. Fig. 3a is an example of the measured relationship between the width of the pattern and the applied pressure for a fixed concentration of PLGA (20%), and a motor speed of 2.5 mm/s. Highly viscous solutions result in the highest pattern resolutions. However, solution viscosities greater than about 400 cp demand high driving pressures to extrude the liquid which may break the tip. Furthermore, highly concentrated solutions evaporate rapidly and plug the tip. For this study, 10 μm structures were deposited at 200 mm Hg using a 20% PLGA solution. Under these optimal conditions, the vertical resolution was found to be about 5–10 μm as shown by SEM analysis. The profile of the structures, which was also measured with an atomic force microscope [26], is not uniform but resembles an elliptical arc with a high aspect ratio. Multilayer structures were fabricated by depositing polymer in successive layers. Figs. 3b and c show a single layer and multilayer structure, respectively.

Microsyringe techniques have been reported elsewhere for use in manufacturing and microelectronics. For example, biocompatible polymers have been deposited using a PAM to realise 3D scaffolds with a resolution of 50 μm [29]. In comparison, our technique, has an improved resolution and can be utilized for biomaterial deposition. The ability to resolve biomate-



(a) (b) (c)

Fig. 3. PAM process parameters and scaffolds: (a) dependence of line width on syringe pressure, (b) 2D PLGA scaffold, and (c) 3D PLGA scaffold.

rial structures on the same length scale as cell and tissue features should enable fundamental studies on the role of the scaffold architecture on tissue function over physiologically relevant parameters. The microsyringe system developed in this study was envisaged as an automated CAD/CAM device, where the operator needs only to input the desired geometry of the scaffold and the machine initiates the microfabrication process. Key advantages of the system are the automated interface, the rapid process time (~minutes) the ease of operation, and the ability to dynamically alter the scaffold (by interrupting the process, altering polymer composition, etc.). The main limitations of this technique arise from the infrastructure investment, the narrow range of viscosities that can be employed to obtain high-resolution structures, and the inability to incorporate particulates for leaching due to plugging of the syringe tip.

3.2. Soft lithography

The soft-lithographic PLGA molding techniques presented in this study were adapted from existing methods to form PDMS replicas. In the literature, PDMS has been used as a stencil [20,23,30], stamp [21,31,32], and microfluidic network [23,27,33–36] to produce patterns of proteins or adhesive ligands. Our innovation was to use PDMS replicas as molds to secondarily microfabricate polymer scaffolds. The advantage of this approach is that a single microfabricated master can be used to produce many PDMS molds that are inexpensive, robust, aseptic, and reusable. The elastomeric properties of PDMS also enable new techniques such as microfluidic molding. One disadvantage of soft lithographic methods is the requirement that scaffolds be continuous structures (i.e. no free-standing structures can be fabricated). In this study, we examined three methods to form biodegradable polymeric scaffolds from PDMS molds.

3.2.1. Micromolding

The micromolding technique is essentially solvent casting on a PDMS mold. Many PLGA scaffolds can be produced with a single PDMS mold. The thickness of the resulting PLGA scaffold is determined by the height of the features on the photolithographic master (~30 μm for this paper) and by the concentration of polymer in the solution. The feature height on the master can in principle be a few microns. However, thin membranes are fragile and are difficult to manipulate manually. We empirically noted that scaffold heights of greater than ~30 μm yielded a scaffold with sufficient integrity to manipulate. The optimal PLGA concentration for use with this method was found to be around 10–15%. At this concentration, the viscosity is high enough (around 100 cp) to allow the polymer solution to permeate the trenches of the PDMS mold when placed under vacuum. It was also observed that the solvent caused the PDMS mold to swell slightly. This limited the lateral resolution to a practical minimum of about 20–30 μm. Finally, we observed that the polymer solution created menisci around each feature resulting in a surface that was not entirely flat. Fig. 4a shows an example of a micromolded PLGA membrane with a line width of 50 μm and the size of the open square region being 300 μm on a side.

Molding of PLGA networks directly from microfabricated masters has been previously reported [24]; however, the technique was not extensively characterized with regard to polymer concentration, mold release conditions, and other process parameters. Furthermore, use of PDMS as an intermediate mold has some advantages that were exploited in this study (see Microfluidic Molding below).

3.2.2. Microfluidic molding

The second variation was based on microfluidic flow. This technique took advantage of the microchannels created when a PDMS mold was reversibly sealed to a

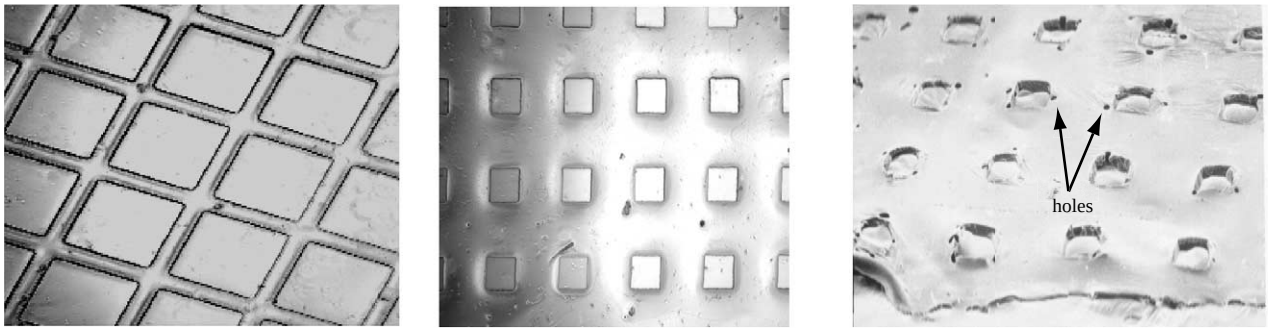


Fig. 4. Scaffolds produced by soft lithography: (a) Micromolding, (b) microfluidics, and (c) spin coating.

Table 1

Method	Lateral resolution (μm)	Vertical resolution (μm)	Optimal PLGA concentration (%)	Advantages	Limitations
Microsyringe	10	5	20	Dynamic control of scaffold Automated, ease of operation Can be used for multilayers	Infrastructure Narrow range of viscosities Cannot use particulates
Soft-lithography				Inexpensive, robust, reusable	Structures must be continuous
Micromolding	20–30	30	10–15	Can be used for multilayers	Uneven surface, manipulation limited
Microfluidic	20–30	10	5–10	Minimal manipulation, flat surface	Limited geometry due to pressure drop
Spin coating	100	7	5	Requires less polymer, thin scaffolds	Small bubbles, uneven surface

substrate. Microchannels were filled with a PLGA solution by application of negative pressure. In theory, the resolution of this technique is only limited by the resolution of the PDMS mold and hence the master. Practically, we found it difficult to mold patterns with small channel dimensions because the negative pressure that must be applied to fill the channels was excessive. Once the PLGA was cured, the PDMS was carefully removed to avoid damaging the thin structures. The average height of the scaffolds we fabricated was 10 μm. A typical scaffold obtained using this technique is shown in Fig. 4b. Unlike the other methods, microfluidic patterning could not be used to fabricate 3D structures because the polymer solution adhered strongly to the underlying substrate and could not be peeled off.

3.2.3. Spin coating

This method utilized a photoresist spin-coater to create a polymer layer that was thinner than the features on the master. We found that a 5% PLGA solution was optimal for this method because the time required to fill the mold was compatible with the spinning time (~30 s). Highly viscous solutions would not completely fill the mold because the solvent evaporated during spinning before the polymer solution could permeate the PDMS structure. With a fixed polymer concentration, the height of the scaffold could be regulated by varying the spinning speed. Typically, a speed of 2000 rpm yielded membranes with an average height of 7 μm. A typical scaffold produced using this method is shown in Fig. 4c. We observed meniscus effects in regions where the polymer was in contact with the hydrophobic PDMS mold, producing non-uniform scaffold heights. We also observed small holes on the membrane surfaces and hypothesize that bubbles were introduced through rapid solvent escape. We observed a practical minimum lateral feature size of 100 μm. Smaller line widths could not be achieved because the polymer solution was not able to fill the narrow microchannels during the spinning process. We also found it difficult to remove membranes composed of large open areas from the mold.

3.3. Comparison of techniques and application to tissue engineering

3.3.1. Comparison of techniques

Table 1 summarizes the features of each technique in terms of resolution, cost and polymer concentration. From this table, we see that the methods differ a number of ways: in lateral resolution where resolution is higher for the PAM system (~10 μm) microns than the soft-lithographic techniques (~25 μm), in vertical resolution (~10 μm) for all methods except micromolding which gives rise to thicker structures, in polymer concentration where the soft-lithographic techniques require only a small volume of polymer with low viscosity, whereas PAM requires a larger volume and high viscosities to obtain high-resolution patterns. In cost the PAM system has substantial infrastructure investment while the soft lithography techniques require the support of a photolithography facility or access to out-sourcing this work to obtain PDMS molds. Both techniques provide the ability to fabricate relatively high-resolution (10–30 μm features) polymer scaffolds for use in tissue engineering research.

3.3.2. Lamination of multiple layers and solvent casting and particulate leaching

Once the membranes were fabricated, 3D structures were assembled by stacking the layers together under an optical microscope using a pair of tweezers and then laminating them. An example of one such structure is shown in Fig. 5a. Porous PLGA molded membranes were easily obtained using all of the soft lithographic methods. The only requirement was that the solution viscosity be low enough so that it can fill the mold evenly, and that the glucose grains be homogeneously mixed. An example of a microporous pattern obtained using micromolding is shown in Fig. 5b.

In summary, our objective was to develop simple, robust microfabrication techniques for the construction of model 2D and 3D biomaterial scaffolds to enable fundamental tissue engineering studies. In this work we have described two methods—one, PAM deposition and

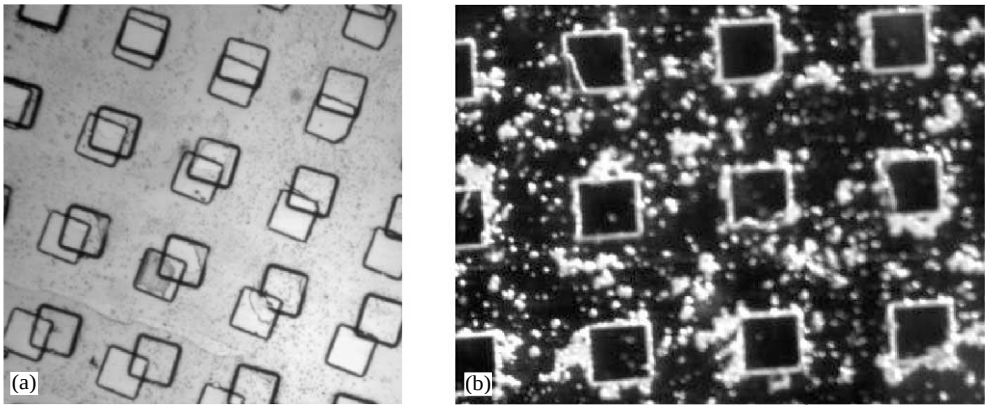


Fig. 5. Applications to tissue engineering I: (a) multilayer PLGA structure formed by thermal lamination of scaffolds produced by micromolding and soft lithography and (b) porous PLGA structure formed by solvent casting and particulate leaching.

the other that utilizes PDMS molding—that have various advantages and disadvantages. PLGA scaffolds were fabricated as a prototypic biomaterial scaffold for tissue engineering. Applications of this technology in tissue engineering were explored by forming multilayer scaffolds and porous scaffolds. In the future, these techniques can be used to study the effect of scaffold architecture on cellular activities such as proliferation, differentiation and motility.

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References

[1] Bhatia SN, Chen CS. Tissue engineering at the micro-scale. Biomed Microdevices 1999;2(2):131–44.
[2] Hadlock T, Sundback C, Hunter D, Cheney M, Vacanti JP. A polymer foam conduit seeded with Schwann cells promotes guided peripheral nerve regeneration. Tissue Eng 2000;6(2): 119–27.
[3] Miller C, Shanks H, Witt A, Rutkowski G, Mallapragada S. Oriented Schwann cell growth on micropatterned biodegradable polymer substrates. Biomaterials 2001;22(11):1263–9.
[4] Li Y, Ma T, Yang ST, Kniss DA. Thermal compression and characterization of three-dimensional nonwoven PET matrices as tissue engineering scaffolds. Biomaterials 2001;22(6):609–18.
[5] Kane RS, Takayama S, Ostuni E, Ingber DE, Whitesides GM. Patterning proteins and cells using soft lithography. Biomaterials 1999;20(23–24):2363–76.
[6] Mikos AG, Lyman MD, Freed LE, Langer R. Wetting of poly(L-lactic acid) and poly(DL-lactic-*co*-glycolic acid) foams for tissue culture. Biomaterials 1994;15:55–8.
[7] Lo H, Ponticiello MS, Leong KW. Fabrication of controlled release biodegradable foams by phase separation. Tissue Eng 1995;1:15–28.
[8] Mooney DJ, Organ G, Vacanti JP, Langer R. Design and fabrication of biodegradable polymer devices to engineer tubular tissues. Cell Transplant 1994;3(2):203–10.
[9] Harris LD, Kim BS, Mooney DJ. Open pore biodegradable matrices formed with gas foaming. J Biomed Mater Res 1998; 42(3):396–402.
[10] Shastri VP, Martin I, Langer R. Macroporous polymer foams by hydrocarbon templating. Proc Natl Acad Sci USA 2000; 97(5):1970–5.
[11] Karamouk E, Mayer J, Wintermantel E, Akaike T. Partially degradable film fabric composites: textile scaffold for liver cell culture. Artif Organs 1999;23(9):881–7.
[12] Rivard CH, Chaput CJ, DesRosiers EA, Yahia LH, Selmani A. Fibroblast seeding and culture in biodegradable porous substrates. J Appl Biomater 1995;6(1):65–8.
[13] Yannas IV, Burke JF, Orgill DP, Skrabut EM. Wound tissue can utilize a polymeric template to synthesize a functional extension of skin. Science 1982;215:174–6.
[14] Zein I, Hutmacher DW, Tan KC, Teoh SH. Fused deposition modeling of novel scaffolds architectures for tissue engineering applications. Biomaterials 2000;23:1169–85.
[15] Mikos AG, Sarakinos G, Leite SM, Vacanti JP, Langer R. Laminated three-dimensional biodegradable foams for use in tissue engineering. Biomaterials 1993;14:323–30.
[16] Giordano RA, Wu BM, Borland SW, Cima LG, Sachs EM, Cima MJ. Mechanical properties of dense polylactic acid structures fabricated by three dimensional printing. J Biomater Sci Polym Ed 1996;8(1):63–75.
[17] Mrksich M, Dike LE, Tien J, Ingber DE, Whitesides GM. Using microcontact printing to pattern the attachment of mammalian cells to self-assembled monolayers of alkanethiolates on transparent films of gold and silver. Exp Cell Res 1997;235(2): 305–13.
[18] Chu TM, Orton DG, Hollister SJ, Feinberg SE, Halloran JW. Mechanical and in vivo performance of hydroxyapatite implants with controlled architectures. Biomaterials 2002;23(5): 1283–93.
[19] McDonald J, Duffy D, Anderson J, Chiu D, Wu H, Schueller O, Whitesides G. Fabrication of microfluidic systems in poly(dimethylsiloxane). Electrophoresis 2000;21(1):27–40.
[20] Folch A, Byung HJ, Hurtado O, Beebe DJ, Toner M. Microfabricated elastomeric stencils for micropatterning cell cultures. J Biomed Mater Res 2000;52(2):346–53.
[21] Kumar A, Biebuyck HA, Whitesides GM. Patterning self-assembled monolayers: application in material science. Am Chem Soc 1994;10:1498–511.

[22] Shive MS, Anderson JM. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev 1997; 28(1):5–24.
[23] Chiu DT, Jeon NI, Huang S, Kane RS, Wargo CJ, Choi IS, Ingber DE, Whitesides GM. Patterned deposition of cells and proteins onto surfaces by using three-dimensional microfluidic systems. Proc Natl Acad Sci USA 2000;97(6):2408–13.
[24] Borenstein JT, Terai H, King KR, Weinberg EJ, Kaazempur-Mofrad MR, Vacanti JP. Microfabrication technology for vascularized tissue engineering. Biomed Microdevices 2002; 4(3):167–75.
[25] Bhatia SN, Yarmush ML, Toner M. Controlling cell interactions by micropatterning in co-cultures: hepatocytes and 3T3 fibroblasts. J Biomed Mater Res 1997;34(2):189–99.
[26] Vozzi G, Previti A, De Rossi D, Ahluwalia A. Microsyringe based deposition of 2 and 3-D polymer scaffolds with a well defined geometry for application to tissue engineering. Tissue Eng 2003;8(6):1089–98.
[27] Zhao B, Moore JS, Beebe DJ. Surface-directed liquid flow inside microchannels. Science 2001;291(5506):1023–6.
[28] Seglen PO. Preparation of isolated rat liver cells. Methods Cell Biol 1976;13:29–83.
[29] Landers R, Mullhaupt R. Desktop manufacturing of complex objects, prototypes and biomedical scaffolds by means of computer-assisted design combined with computer-guided 3D plotting of polymers and reactive oligomers. Macromol Mater Eng 2000;282:17–21.
[30] Ostuni E, Kane R, Chen CS, Ingber DE, Whitesides GM. Patterning mammalian cells using elastomeric membranes. Langmuir 2000;16(20):7811–9.
[31] Wilbur JL, Kumar A, Biebuyck HA. Microcontact printing of self-assembled monolayers: application in microfabrication. Biomaterials 1995;18:2216–21.
[32] Chen CS, Mrksich M, Huang S, Whitsides GM, Ingber DE. Geometric control of cell life and death. Science 1997;276: 1425–8.
[33] Beebe DJ, Moore J S, Yu Q, Liu RH, Kraft ML, Jo BH, Devadoss C. Microfluidic tectonics: a comprehensive construction platform for microfluidic systems. Proc Natl Acad Sci USA 2000;97:13488–93.
[34] Delamarche E, Bernard A, Schmid H, Michel B, Biebuyck H. Patterned delivery of immunoglobulins to surfaces using microfluidic networks. Science 1997;276(5313):779–81.
[35] Patel N, Padera R, Sanders GH, Cannizzaro SM, Davies MC, Langer R, Roberts CJ, Tendler SJ, Williams PM, Shakesheff KM. Spatially controlled cell engineering on biodegradable polymer surfaces. FASEB J 1998;12(14):1447–54.
[36] Folch A, Ayon A, Hurtado O, Schmidt MA, Toner M. Molding of deep polydimethylsiloxane microstructures for microfluidics and biological applications. J Biomech Eng 1999;121(1):28–34.