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Microfabricated PLGA scaffolds: a comparative study for application to tissue engineering

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Abstract

A variety of techniques for the manufacture of biodegradable, three-dimensional scaffolds for tissue engineering have been developed in recent years. In this study, we report and compare two simple methods for fabricating poly(DL-lactide-co-glycolide) (PLGA) scaffolds with feature sizes of 10–200 μm, which have been developed in our laboratories. The first technique is based on the use of a microsyringe that makes use of a computer-controlled, three-axis micropositioner, which allows the control of motor speeds and position. A PLGA solution is drawn from the needle of the syringe by the application of a constant pressure of 10–300 mm Hg resulting in controlled polymer deposition of 10–600 μm in diameter. The second technique is based on "soft lithographic" approaches that utilizes a Poly(dimethylsiloxane) (PDMS) mold. The polymer solution is cast on the mold under vacuum. Polymer concentration, solvent composition, and casting conditions influence the integrity and the lateral resolution of the resulting scaffold. Both techniques allow the possibility of constructing three-dimensional architectures that permit the study of cell behaviour in an environment similar to that in vivo, and may provide tools for the construction of engineered tissue. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Microfabrication; Microsyringe; Micromolding; Soft lithography; PLGA; Tissue engineering

1. Introduction

Control of microscale polymer scaffold architecture is of fundamental importance in tissue engineering for emulating the microscale structure of tissues in vivo [1,2].

A wide variety of techniques for controlling the architecture of biodegradable polymers such as poly-(DL-lactide-co-glycolide) (PLGA) are already available for relatively large feature sizes of the order of millimeteres or centimeters. These include polymer extrusion [3], solution casting and particulate leaching [4], deposition of a polymer solution stream on a spinning mandrel, and manipulation of sheets of polymer meshes. To achieve arbitrary three-dimensional geometries, preformed sheets of biomaterial have been cut and laminated with a resolution of 0.5 mm [5].

To manipulate scaffold architecture on smaller length scales, injection molding against a microfabricated silicon template was utilized by Kapur et al. [6] with a resolution of 10 μm. In addition, a 3D-printing technique developed by Park et al. [7] utilizes a polymer powder spread on a plate. Three-dimensional structures are achieved by application of a solvent binder through an inkjet head [8]. The resolution of this system is dependent on the polymer particle size where typical features are on the order of 300 μm. Solid freeform microfabrication of polymers and gels based on fluid dispensing and extrusion methods have been used in several areas of engineering, in particular by Calvert et al. [9] and Lombardi et al. [10]. More recently, similar techniques have been applied by others in the biomedical field [11,12]. In Ref. [11], a large-bore syringe is used to deposit polymers underwater. The method is claimed to achieve a resolution of the order of 50 μm and necessitates the use of elevated pressures (several bars) to extrude the polymer. Fused deposition modelling, described in Ref. [13], utilises a heated barrel to extrude thermoplastic polymers.

While these techniques are useful for certain applications, many of them require processing conditions such as heating, high pressure and polymer grinding that may be limiting for the inclusion of bioactive moieties or high-resolution features, respectively. In this study, we explored two techniques for controlling PLGA architecture at the microscale: microsyringe deposition and soft lithographic

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micromolding. Soft lithography is a well known microfabrication technique, pioneered by Whitesides et al. [14–17]. In most cases the elastomeric membrane is used as a stamp or a stencil [18,19]. The micromolding method has been applied to PLGA, which cannot be stamped or stencilled in the usual manner owing to its high viscosity. Variations of this technique have been reported previously for fabrication of non-degradable polyurethane scaffolds [20] and continuous PLGA membranes [21]. In this study, we use soft lithography and a pressure-driven microsyringe to obtain discontinuous, biodegradable scaffolds to form two- and three-dimensional structures for tissue engineering. We compare and contrast the two techniques described here with regard to optimal polymer concentration, cost, and resolution.

2. Materials and methods

The polymer solution is obtained by dissolving 85/15 poly (DL-Lactide-co-glycolide) (PLGA) (Birmingham Polymers, Birmingham, AL, USA) in chloroform. The concentration of solutions varied between 5% and 20% according to the microfabrication technique used.

3. Mycrosyringe deposition

The first fabrication technique, developed at Interdepartmental Center of Research "E. Piaggio" at the University of Pisa, is based on the use of a microsyringe that allow the deposition of a wide range of polymers [2]. Fig. 1A illustrates the main features of the syringe based system. This method makes use of a three-axis micropositioner (Ealing, London, UK) with a precision of 0.1 μm. The controller of the stepper motor micropositioner is interfaced to a PC which enables the control of motor speed and position through the use of in house software. The syringe is mounted on the vertical (*z*) axis of the positioner while the substrate (a glass slide) is mounted on the horizontal motors and moves relative to the syringe. A Peltier thermoregulator is integrated into the horizontal motors for temperature control to modulate solvent evaporation. The microsyringe is a stainless steel barrel with a 10- to 20-μm o.d. capillary tip held in place by an o-ring. The barrel of the syringe is filled with a viscous solution of PLGA in chloroform (about 12% w/v), which is expelled by filtered compressed air at a constant low pressure. In this way, the solution contacts only steel, glass and the o-ring, and contamination is minimized. A software regulated system of valves and sensors maintains and controls the pressure to within 1 mm Hg. By varying the applied pressure, the speed of the motors, and the solution viscosity, a wide range of patterns with lateral dimensions ranging from 10 to 300 μm can be microfabricated. Using the *z*-axis of the micropositioner, three-dimensional scaffolds are obtained by depositing successive layers on top of each other.

4. Micromolding by soft lithography

The other technique, developed at the Microscale Tissue Engineering Laboratory, University of California, San Diego, is based on "soft lithographic" approaches that utilize a poly(dimethylsiloxane) (PDMS) mold and is depicted in Fig. 2. For this technique, the first important step is the production of the silicon template that allows the fabrication of the PDMS mold. The details for the microfabrication of the master have been previously described [1]. Briefly, wafers were spin-coated with EPON-SU8 photoresist (Microchem, Newton, MA), baked, then exposed to ultraviolet light in a Bottom Side Mask Aligner (Karl Strauss, Waterbury Center, VT) through a high-resolution transparency mask, drawn with Coreldraw 9.0 and printed using a commercial Linotronic-Hercules 3300 dpi high-re-

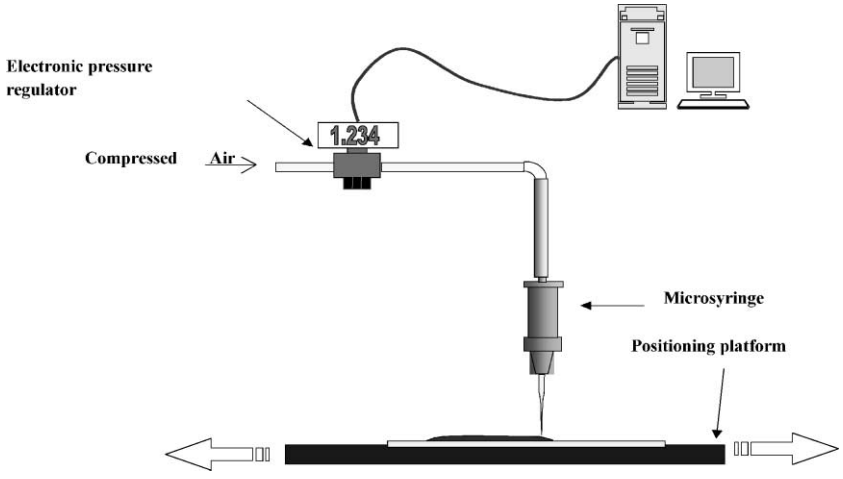

Fig. 1. Principle of action of the microsyringe.

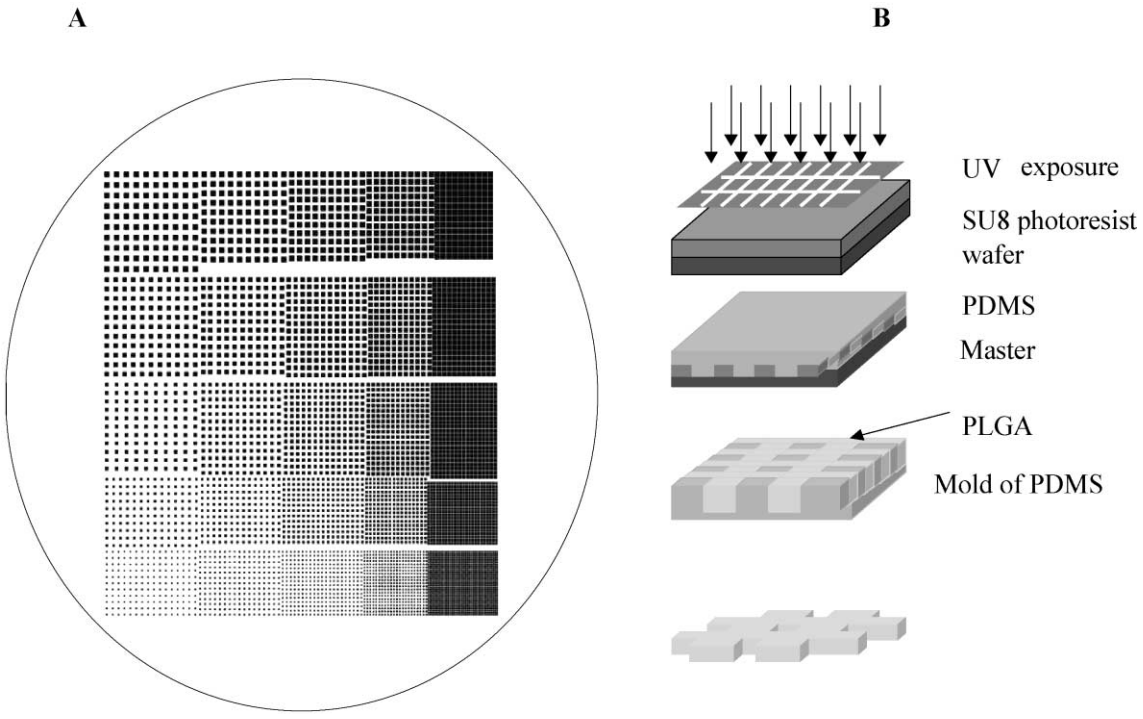

Fig. 2. (A) An example of a mask used in micromolding. (B) Schematic illustration for the fabrication of a PLGA pattern with the micromolding technique [see text for details].

solution line printer. Exposed photoresist was then developed (SU8 developer, Microchem) and the wafers were baked again.

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 bubbles created by pouring, and then baked in an oven for 2 h at 65 °C. After cooling and demolding the PDMS mold was then washed with 70% ethanol and sonicated for 5 min. Once the PDMS master mold was obtained, the PLGA solution was cast on the mold and placed under vacuum for 2 min to allow the polymer to fill the microchannels present in the mold and displace any air present. Excess PLGA was removed by passing a glass slide across the top of the mold, and the PDMS mold and polymer were baked for 30 min at 60 °C. After cooling, the PLGA pattern was peeled off with a pair of tweezers. It is also possible to construct laminated three-dimensional structures by applying a mechanical load to a set of PLGA patterns stacked together and heating for 10 min at 60 °C.

5. Results and discussion

The importance of biomaterial architecture at the microscale in Tissue Engineering has been recognized in recent years [1,2,22,23]. While several different techniques have been developed to control scaffold architecture, each method has intrinsic limits related to the resolution, necessary infrastructure, or versatility. We have developed two techniques in our laboratories that enable the fabrication of many different kinds of biomaterial patterns with controlled geometry: microsyringe deposition and soft lithographic micromolding.

The microsyringe deposition method is schematically depicted in Fig. 1A. In this system, a variety of patterns with different geometries and widths can be obtained. This versatility is useful for the study of optimal scaffold topology to promote desired cellular behaviour. The minimum line width obtainable is limited by the dimensions of the syringe tip, and is of the order of 10 μm. Smaller capillaries are too fragile to be employed and require excessively high driving pressures. A wide range of polymers can be used, the only requirement being solubility in a volatile solvent, although solutions with a high viscosity (about 200 Cp), which do not spread quickly, are necessary in order to achieve high resolution. The width and profile of the pattern depends on the dimensions of the needle, its distance from the substrate, the concentration of the polymer solution, the applied pressure and the motor speed [2] and can be finely modulated by changing any of these parameters. The system is user-friendly and user intervention is minimal. Once the geometry of the scaffold has been chosen and input to the PC, and the barrel of the syringe has been filled with the given solution, the only task of the operator is to change the substrate. Fig. 3A shows an optical micrograph of a two-dimensional PLGA scaffold created with this technique. Fig.

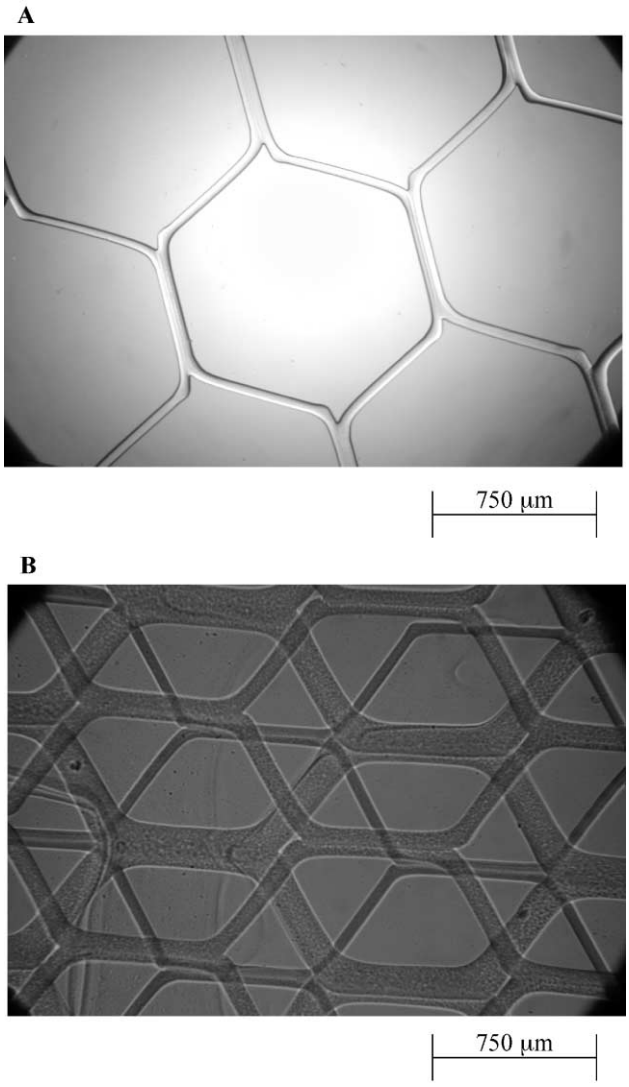

Fig. 3. (A) A typical 2D polymer pattern and (B) an example of a 3D scaffold of variable line-width realised with the microsyringe technique.

3B illustrates how three-dimensional scaffolds can be constructed by fabricating successive layers and using the *z*-axis of the micropositioner to control the height of the syringe tip. The line width within each layer was varied by modulating the applied pressure.

The soft lithographic micromolding method also allows the fabrication of patterns with micron-scale geometry. Using a microfabricated template, one can produce numerous patterns of PDMS with a single desired geometry. Each of these PDMS molds can be used to fabricate many PLGA scaffolds. The polymer scaffold can be peeled off the mold and manipulated with ease when set. This technique is less versatile than the microsyringe technique in that the architecture cannot be varied arbitrarily by the user; however, once a mold has been realised, the scaffold can be replicated several times without the aid of specialised facilities. Using inexpensive transparencies as photolithographic masks, the mask resolution is approximately 20 μm and the resolution of the micromolding technique is about 30 μm due to slight swelling of the mold in the polymer solvent (Fig. 4A). We expect that the use of higher resolution chrome/quartz masks would improve the resolution of the molding technique by an order of magnitude; however, the cost of the technique would also increase considerably. The thickness of the pattern is determined by the height of the features on the photolithographic master and by the concentration of the polymer solution—in these experiments approximately 30 μm. Finally, due to the contact angle of the polymer solution with the hydrophobic PDMS mold, the top surface is not flat but is modified by a meniscus at each polymer/PDMS interface.

The optimal polymer concentration is around 10%–15%. At this viscosity of approximately 100 Cp, the solution can flow spontaneously through the microchannels in the PDMS mold. Using the micromolding technique, multilayer structures can be fabricated by manual alignment of individual

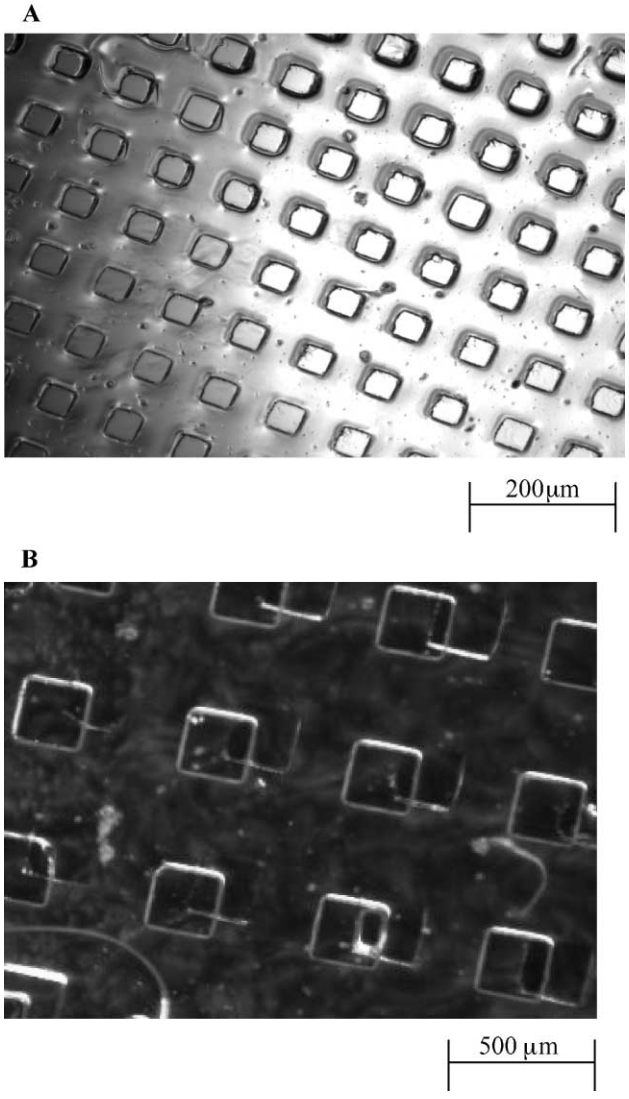

Fig. 4. (A) A 2D pattern realised using micromold and (B) a 3D pattern obtained by laminating micromolded layers.

layers followed by thermal fusion, as described in Ref. [12]. 2D and 3D structures are reported in Fig. 4A and B, respectively.

6. Conclusions

In summary, our objective was to develop and explore novel microfabrication techniques for the microfabrication of biocompatible, bioerodable, discontinuous polymer scaffolds. To this end, we developed two novel methods which can be used to construct PLGA scaffolds with many potential geometries, such as grids, hexagons and dendritic structures. These methods offer a valid alternative to the techniques currently being used to microfabricate tissue engineering scaffolds. Their lateral resolution of approximately 10 μm is comparable or superior to that of other commonly employed methods. Control of scaffold architecture at the microscale will find applications in fundamental studies of cell fate and function in tissue engineering. Furthermore, both techniques have been utilized to construct multilayer, three-dimensional scaffolds that will permit the study of cell behavior in microenvironments that approach the complexities found in vivo. Finally, these techniques may find applications in other areas of microtechnology such as microactuators, microfluidics, and integrated microdevices.

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