Microsyringe-Based Deposition of Two-Dimensional and Three-Dimensional Polymer Scaffolds with a Well-Defined Geometry for Application to Tissue Engineering*

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ABSTRACT

A technique for controlled deposition of biomaterials and cells in specific and complex architectures is described. It employs a highly accurate three-dimensional micropositioning system with a pressure-controlled syringe to deposit biopolymer structures with a lateral resolution of 5 μm. The pressure-activated microsyringe is equipped with a fine-bore exit needle and a wide variety of two- and three-dimensional patterns on which cells to be deposited can adhere. The system has been characterized in terms of deposition parameters such as applied pressure, motor speed, line width and height, and polymer viscosity, and a fluid dynamic model simulating the deposition process has been developed, allowing an accurate prediction of the topological characteristics of the polymer structures.

INTRODUCTION

Most types of tissue that are now in advanced stages of engineering are either amorphous or isotropic (such as bone and cartilage) or have a planar structure (the skin and blood vessels). However, some of the more complex organs and tissues, such as the liver, heart, and neural tissue, are proving more difficult to engineer because they have a specific three-dimensional cell distribution in which the three-dimensional structure is inextricably linked to its function. For this type of tissue, it is considered essential to have a method of creating biomaterial scaffolds having a known and well-defined topology.

An important example of structurally organized tissue is neural tissue, such as the retina. The retina has a modular architecture and is made up of three main layers separated by interweaving layers of cell processes. Within their layers neurons of the same type commonly form nonrandom planar arrays known as mosaics.1 The correct topographic development of a single layer of cells leads to correct connections between each layer, which then leads to a functional retina. Another example of a tissue with a well-defined architectural organization is the liver, in which hepatocytes form a close packed hexagonal structure interlaced with blood vessels and bile ducts. The efficient transport, exchange, and collection of nutrients and wastes depend enormously on the exquisite organization, at the cellular level, of the liver.

Several methods for the deposition of biopolymers with controlled architecture have been described in the literature. The most well known, perhaps, is that pioneered by the Mechanical Engineering Group at the Massachusetts Institute of Technology (MIT, Cambridge, MA),2 known as 3DP (3-dimensional printing). In this method the polymer powder is spread on a plate, and the solvent or binder is sprayed by an ink-jet head onto the powder. The ink-jet head is machine driven and raster...
scans the plates, depositing binder wherever necessary. Where the binder lands, the powder binds. Successive layers can be built up in this way, and have been used to fabricate 3-D scaffolds for the adhesion of hepatocytes.\textsuperscript{3} It does, however, have a fairly low resolution; for example, about 300-\(\mu\)m structures of polylactic acid can be patterned.

Another well-known method is that developed by the Whitesides group at Harvard University (Boston, MA).\textsuperscript{4} This method, called soft lithography, is based on polydimethoxysilane stamps and self-assembly to produce high-resolution structures, with depths of a few microns. So far, this method has been used successfully to produce cell patterns using sophisticated surface chemistry, but it is limited to low-depth 3-D.

Another method reported by Odde and Renn is that of laser-directed guided writing.\textsuperscript{5} In this method, protein and small biomaterial particles can be made to assemble in particular locations, using a technique similar to laser tweezers.

The CAD/CAM (computer-aided design/computer-aided manufacturing) approach developed at Carnegie Mellon University (Pittsburgh, PA)\textsuperscript{6} uses sheets of pre-formed biomaterial and stacks them vertically to make 3-D structures. Because the current resolution is only 0.5 mm by 12 mm in diameter, it is not entirely suited to controlling the fine structure of a scaffold.

In this article we present a novel method for the deposition of biopolymers in high-resolution structures, using a pressure-activated syringe. It is a highly versatile instrument, not just for its potential application to tissue engineering, but also to study cell motility, organization, and cell reaction to various topographies. The present work is focused on the characterization of the deposition system and on the patterns produced using a variety of polymers at different concentrations.

**MATERIALS AND METHODS**

**Polymers**

Two different polymers were used: poly-l-lactic acid (PLLA, MW 300,000; Boehringer Ingelheim, Ingelheim, Germany) and polycaprolactone (PCL, MW 65,000; Sigma, St. Louis, MO). The biopolymers employed have been studied by our group in the form of spin-coated films and their surface properties (surface charge density, dielectric constant, morphology, and contact angle) and their suitability for cell adhesion have been evaluated.\textsuperscript{7} The polymers were dissolved in chloroform to give 1, 2, and 3\% (w/v) solutions of PLLA and 10, 15, and 20\% (w/v) solutions of PCL. PLLA was also blended with PCL to provide additional carboxyl groups to permit surface derivatization in ensuing studies. A blend of 2.5\% PLLA and 20\% PCL (w/v), prepared by mixing equal quantities of 5\% PLLA and 40\% PCL, was found to be optimal for syringe deposition.

To provide input concerning the parameters of the fluid dynamic model, the viscosity of each solution was measured with a Saybolt viscosimeter. Measured viscosities are tabulated in Table 1.

**Deposition system**

The deposition system utilized consists of a stainless steel syringe with a 20-\(\mu\)m glass capillary needle as the tip. A home-built vertical puller was used to pull the tips, which were prepared from soda glass hematocricc capillaries (Globe Scientific, Paramus, NJ) with an outer diameter of 1.5 mm and an inner diameter of 1.15 mm. As shown in Fig. 1, the tip has a flat end and is gently tapered, a characteristic that lends itself to streamlined flow in the narrowest parts of the capillary. Each capillary is pulled under the same conditions and the internal diameter of the tips is 20 \(\pm\) 2\(\mu\)m. The two tips obtained from each capillary tube pulled are not symmetrical, as one is slightly longer than the other. The needle is connected to the syringe barrel and held in place by a small O ring. A solution of the polymer is placed inside the syringe, which has a capacity of about 5 ml.

The syringe, which has no plunger but is driven by filtered compressed air at a pressure of about 10–500 mmHg, is attached to the vertical \(z\) axis of a three-axis stepper-motor micropositioning system (Ealing, UK) with a resolution of 0.1\(\mu\)m. A planar substrate, generally a 3 \(\times\) 3 cm glass slide, is fixed on the horizontal \(x\) and \(y\) axes of the micropositioner and is made to move under the syringe during deposition. When pressure is applied to the syringe, tiny amounts of polymer ooze out through the tip. If the needle is too far from the surface, the polymer solidifies on the tip, preventing further deposition. However, if the tip is “drawn” along the substrate, keeping it just high enough that it does not contact the substrate and break, fine lines of polymer are traced on the substrate. Precise measurements of the optimum height of the tip with respect to the substrate have

**Table 1. Viscosities of Solutions**

<table>
<thead>
<tr>
<th>Polymer solution</th>
<th>Viscosity (cP)</th>
</tr>
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<tbody>
<tr>
<td>PCL</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>20%</td>
</tr>
<tr>
<td>PLLA</td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>3%</td>
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<tr>
<td>Blend</td>
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not been made; we estimate it to be on the order of the tip diameter. In preliminary experiments, it was ascertained that the most reproducible technique for obtaining smooth, even, microsized patterns was to apply a low and constant pressure (rather than a pulsed pressure) to the syringe and to allow the polymer to be dragged across the surface of the substrate, much like writing with an ink pen. Moreover, it was established that the concentration of polymer must lie within an optimal range, between about 100 and 700 cP. Low-viscosity solutions leak out of the tip whereas highly viscous solutions require pressures that may damage the tip and be a danger to the operator. A schematic illustration of the pressure-controlled deposition system is shown in Fig. 2.

FIG. 1. Photograph and optical micrograph (inset) of the capillary tip. Original magnification (inset): ×10.

FIG. 2. Schematic illustration of the pressure-controlled deposition system.
The entire system including valves, pressure sensors, and position controllers is interfaced to and controlled by a personal computer through a GPIB (general programming interface bus) card. Appropriate software to drive the system, written in C language, allows simple patterns such as lines; rectangular, hexagonal, or triangular grids; and spirals to be deposited. More complex patterns, such as dendritic structures, are easy to design and have been incorporated into the software.

The pressure sensor and associated control system allows the applied pressure to be controlled to within ±5 mmHg, in the range of 10 to 1000 mmHg, and the velocity of the substrate with respect to the syringe to be varied between 0.5 and 2.5 mm/s. Each polymer concentration was deposited at different applied pressures and $x$, $y$ motor velocities in order to determine a range of optimum operating conditions.

**Pattern depositions and characterization**

Simple 2- and 3-D patterns of the polymers at various concentrations were deposited at different pressures and deposition speeds. In particular, a series of connecting hairpins of varying dimensions were fabricated under different conditions and analyzed with an optical microscope (AX 70; Olympus, Tokyo, Japan) to determine the width of deposited lines and the distance between adjacent lines. The mean width was found by averaging measured widths over each line deposited, and the measurement accuracy was ±1 μm. At least six measurements of line width and distance between lines were made for each sample. Atomic force microscopy was used to measure the profiles of the patterns.

**MODEL**

Complex models such as those used in dynamic wetting systems can be used to predict the line width and...
height of the patterns deposited. These models use the dynamic contact angle, which requires flow visualization techniques to be measured, to describe the equilibrium configuration of a liquid in a coating system.\textsuperscript{9} As a first approximation, we developed a simple fluid-dynamic model that enables the prediction of the width and height of the patterns. The model is focused on the conditions at the tip of the needle, at the point where the polymer exits.

We assume that there is a simple geometry at the tip, and that the polymer solution does not change dimensions as the solvent evaporates. As shown in Fig. 3, the forces in play at the tip where the fluid is expelled are as follows:

- Driving pressure $P$ and the weight of the polymer in the syringe barrel
- Additional pressure $P^* \text{ due to vapor pressure of the solvent}$
- Surface tension between polymer solution and air, $\gamma$
- Dynamic friction between fluid and glass, which is a function of the viscosity, $\mu$, of the solution

If the balance of all forces and energies at the tip of the syringe is considered, a multivariable system of equations with an infinite number of solutions is obtained. To simplify the model, we assume that the driving pressure is the predominant force in this system and the other forces are negligible.

The flow of polymer from the needle is:

$$Q = \frac{dV}{dt} \quad (1)$$

where $V$ is the volume of polymer deposited and $t$ is time. According to the atomic force microscopy (AFM) measurements, reported in the following section, the profiles of the lines can be approximated to an elliptical segment. Given the high aspect ratio (ratio of line width to height), the product of height and width can be used to estimate the cross-sectional area of the deposited structures. Thus we can approximate the flow as

$$Q = ah\frac{dl}{dt} = ahv_0 \quad (2)$$

where $a$ is the line width and $h$ is the height of the polymer pattern, $l$ is the length of polymer deposited in time $t$, and hence $v_0$ is the velocity of the substrate with respect to the syringe. If we assume streamlined flow, and take the polymer to be a viscous Newtonian fluid, the flow inside the capillary is given by Poiseuille’s equation,

$$Q = \frac{\pi R_s^4}{8\mu} \frac{dp}{dz} \quad (3)$$

where $R_s$ is the internal radius of the tip of needle, $dp/dz$ the applied pressure gradient, and $\mu$ is the viscosity of the polymer.

Substituting Eq. (2) into Eq. (3), and rearranging, an expression for the line width $a$ can be obtained:

$$a = \frac{\pi R_s^4}{8\mu v_0 h} \frac{dp}{dz} \quad (4)$$

Before the fluid exits the tip, a certain critical pressure, $P_{\text{crit}}$, proportional to the viscosity of the solution, must be applied. Below this critical threshold deposition cannot occur because the frictional forces are greater than the driving pressure. The pressure gradient in the syringe is negligible in the widest part of the syringe, and is maximum in the tapered region of the tip. In this model, $dp/dz$ has been approximated to $(P + P_{\text{crit}})/h_z$, where $P + P_{\text{crit}}$ is the applied driving pressure and $h_z$ is the length of the tapered zone of the capillary. Equation (4) can then be expressed as

![FIG. 5. Hexagonal grids of the PCL–PLLA blend. The motor speed was 2.5 mm/s. (a) Hexagons, with sides of 500 μm, deposited with increasing driving pressure (30 to 60 mmHg) in the direction of the arrow. (b) Hexagons with sides of 250 μm; driving pressure, 70 mmHg. Original magnification: ×1.25.](image-url)
The experimental pressure-versus-line width data for the polymers deposited were fitted to Eq. (5). Values of viscosity used in the model were interpolated from the experimental data in Table 1, and \( h \), the height of the polymer structures, was obtained by averaging the AFM data for each polymer concentration. Because \( h \) varies between 11.8 and 9.2 mm from tip to tip, an average value of 10.5 mm was used in the numerical calculations.

For a given profile aspect ratio, the model can be used to predict line width, \( a \), as a function of applied pressure \( P_0 \), motor velocity \( v_0 \), and polymer viscosity \( \mu \). If the profile of the pattern has not been measured, the width-to-height ratio of the deposited lines can be estimated from an experimental plot of driving pressure against line width by recursive means, assuming a rectangular profile.

\[
a = \frac{\pi R_s}{8\mu v_0 h} \left( \frac{P + P_{cr}}{h_z} \right)
\]

(5)

**RESULTS**

**Geometric analysis of deposited patterns**

Various types of pattern were deposited, for example, Fig. 4 shows a square grid of the PLLA–PCL blend with sides of 500 \( \mu m \), deposited at a pressure of 50 mmHg, and a motor velocity of 2.5 mm/s. To reproduce the hexagonal organisation of hepatic and retinal tissue, we also deposited hexagonal grids, in which the sides of the hexagons were varied in length. Figure 5a and b are optical micrographs of the hexagonal grids of the PCL–PLLA blend with sides of 500 and 250 \( \mu m \), respectively. In Fig. 5a, the driving pressure is increased from 30 to 60 mmHg during deposition to demonstrate the effect of pressure on line width.

To analyze the relationship between the width and height profile of the polymer patterns and the controllable experimental parameters, namely applied pressure, polymer concentration, and motor speed, a series of connecting hairpins were deposited and each parameter was

**FIG. 6.** Hairpin structures of (a) 20% PCL, (b) 2% PLLA, and (c) PLLA–PCL blend. Increasing driving pressure (in the direction of the arrows) is applied during deposition. The range of pressures (in mmHg) used are shown. Original magnification: \( \times 1.25 \).
varied in turn. Figure 6a–c illustrates the hairpin lines of 20% PCL, 2% PLLA, and the PCL–PLLA blend, respectivly. All lines were deposited at a constant motor speed of 2.5 mm/s, while the pressure was increased by 5 mmHg every two lines. The ability to finely modulate line width by applying small variations in pressure is evident. In Fig. 7, height profiles as obtained from AFM measurements of 3% PLLA, are reported. All the polymers were observed to have profiles approximating a squashed elliptical arc, which decreased in roughness as the pressure was increased. The height of each polymer deposited did not change appreciably with increasing pressure, particularly in the case of the PLLA solutions (Fig. 8), so for the purpose of the model the height was taken to be constant and independent of pressure for a given concentration.

Figure 9a–c shows the measured line widths of solutions of PCL, PLLA, and the PCL–PLLA mixture, respectively, as a function of applied pressure for each concentration. The fitted data obtained from the model are also shown.

Line widths as a function of the deposition velocity for PCL are plotted in Fig. 10. As predicted by the model, line width is inversely proportional to deposition velocity. At present, with a maximum motor velocity of 2.5 mm/s, the minimum line width obtained was on the order of 10 to 20 μm. In principle, the line width can also be decreased by using faster motors. The y intercept of the plots can be used to estimate the minimum line width expected for a given deposition speed, although given the limitations of the model at low pressures, this value can only be taken as indicative.

Three-dimensional structures

We also attempted to make three-dimensional structures by depositing a rectangular or hexagonal grid, allowing it to dry thoroughly, and then depositing another on top. This was done for several layers. On subsequent examination with a scanning electron microscope, it appeared that the layers had fused into one another, indicating that the solvent in overlying layers penetrates and dissolves those underneath, thus producing a dense and compact monolithic structure.

An alternative method was also tested, in which rectangular grids were deposited, and then stacked together and held in place by gluing the corners with chloroform.
FIG. 9. Line widths of (a) PCL, (b) PLLA, and (c) PLLA–PCL blend at different concentrations as a function of applied pressure. The points refer to experimental data, and the lines pertain to the model. Deposition speed: 2.5 mm/s. Deviations between individual samples were on the order of $\pm 5 \, \mu m$ for all pressures.
resulting structure, individual layers were distinguishable; however, this technique is not strictly three-dimensional microfabrication, as part of the fabrication process is manual. To obtain truly three-dimensional microstructures, it is necessary to separate adjacent layers with an inert yet easily removable material during the deposition process. This was achieved by depositing layers of polymer interleaved with layers of a water-soluble polymer deposited with a second syringe. Using this technique, three-dimensional structures of PLLA, as shown in Fig. 11, were obtained. These structures are currently under investigation.

DISCUSSION

As demonstrated by the graphs and photographs in Figs. 4 to 10, the syringe-based deposition method described is capable of fabricating microscale structures of polymers in a wide variety of patterns and with a wide range of thicknesses. This versatility is useful for the study of optimal scaffold topology to promote desired cellular behavior. The operating system is user friendly and does not require special skills. Once the geometry of the scaffold has been chosen and input to the PC and the syringe is filled with a few milliliters of solution, several scaffolds can be microfabricated in the space of 1 h (to make a 1-cm² two-dimensional hexagonal scaffold as shown in Fig. 5 takes about 6–7 min).

Figures 8 and 9a and b illustrate that for a given pressure, both the height and width of the pattern are inversely proportional to the concentration. At high viscosities or concentrations, the volume flow rate for a fixed pressure is low, so as expected, the dimensions of the lines are reduced. This can also be inferred from Eq. (5). To achieve high resolutions, it is thus necessary to use viscous solutions with low flow and spreading rates. The minimum line width obtainable is limited by the dimensions of the syringe tip, and is on the order of 20 μm. Smaller capillaries down to about 5 μm could be used to produce structures with a resolution of 5–10 μm, although they require higher driving pressures and great care must be taken to avoid tip breakage.

Any type of polymer or polymer blends can be utilized
for microsyringe deposition; the only requirements are that the polymers be soluble in a volatile solvent and that the solutions have a viscosity between 100 and 700 cP. The balance between viscous forces, which cause the fluid to shear, and surface tension forces, which tend to minimize the surface area of the fluid, is given by the capillary number.\textsuperscript{9}

\[
C_a = \frac{\mu V_0}{\gamma}
\]

The capillary number can be used to distinguish between the two flow regimes. Highly fluid solutions, of viscosities less than 100 cP, tend to drip out of the syringe tip as discrete drops even without the application of pressure because the capillary number is low and fluid flow is dominated by surface tension effects. More viscous solutions, on the other hand, have high capillary numbers and fluid flow is due to the applied pressure gradient. The upper limit of viscosities was determined principally by safety considerations.

Albeit highly simplified, and despite several physical assumptions, the model faithfully reflects the experimental results in the range of pressures tested and can be used to predict the geometric parameters of any polymer deposited by this technique. A major limitation of the model is that it requires \textit{a priori} knowledge of the profile of deposited structure. However, the profile can be easily predicted from a pressure-versus-line width plot, as shown in Fig. 9a–c. Because all forces except the force due to the driving pressure were ignored, the model is not accurate at low values of driving pressure, where surface tension and other forces may be competitive. In fact, the critical pressure required to expel fluid from the needle, \(P_{\text{crit}}\), was calculated by extrapolating the model to the \(x\) intercept. In practice, the minimum pressure required to initiate deposition was in the range of 10 to 30 mmHg for all solutions, whereas \(P_{\text{crit}}\), according to the model, ranges from 1 to 20 mmHg depending on the viscosity of the solution.

\section*{CONCLUSIONS}

The syringe-based fabrication technique described here is based on a pressure-controlled 2- and 3-D deposition system. The main advantages of this technique over other scaffold fabrication methods are its simplicity and the possibility of modulating line widths and heights over a wide range by varying the polymer concentration or viscosity, applied pressure, and deposition speed. Other applications of this technique have been proposed in the field of actuator microengineering,\textsuperscript{10} and demonstrate the universality and flexibility of the technique. A simple fluid dynamic model has been developed to describe the physical dimensions of the fluid as it is expelled from the syringe tip. The model enables the prediction of essential topological parameters of the structures deposited.

Future investigations into the feasibility of this deposition technique for tissue-engineering purposes will be focused on improving and demarcating cell adhesion on three-dimensional stacked structures.

\section*{ACKNOWLEDGMENTS}

The authors thank G. Tarone and G. Basta for providing cells, C. Ascoli and P. Baschieri for the AFM scans, and above all Prof. Mario Pellegrino for the inexhaustible supply of tips.

\section*{REFERENCES}


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