BIOERODIBLE SCAFFOLDS FOR IMPLANTABLE MICROFLUIDIC PROBES IN CONVECTION ENHANCED NEURAL DRUG DELIVERY

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Abstract: Convection enhanced delivery (CED) is a promising new approach that can be used for local delivery of biological agents in the brain. In CED drugs are infused directly into tissue through a needle or catheter. In this study we developed a flexible microfluidic device for CED constructed from a biocompatible polymer (parylene C). To allow this device to be inserted into tissue we developed a rigid poly(DL-lactide-*co*-glycolide) scaffold to support the flexible probe during insertion. The device was found to be easily insertable into agarose gel brain phantoms and was capable of delivering solutions into the gels with little backflow along the outside of the probe, which often limits infusion rates through larger needles. The *in vitro* degradation time of the scaffolds was examined and found to be 3 – 4 weeks, which is consistent with the previous reports. The effect of the scaffold on flow through the microfluidic device was examined and found to be negligible. The rigid scaffold facilitates implantation of flexible microfluidic devices, making them viable for use in chronic CED protocols.

Introduction

Convection enhanced delivery (CED) is a promising new technique for treating neurological disorders. In CED drugs are infused directly into tissue through a needle or catheter [1]. This technique circumvents the blood brain barrier and achieves high local concentrations of drug with fewer side effects than with systemic delivery. While direct, local delivery in the central nervous system (CNS) has been achieved in the past by using drug-loaded polymer disks , which deliver drugs over time via diffusion [2], many drugs that are delivered directly to the brain are subject to rapid elimination from the interstitial space via permeation through the capillaries or metabolism within brain tissue [3]. Because of this rapid removal, drugs delivered by diffusion are often only able to penetrate a small distance (approximately 2-4 mm [2], [4]) from the implant, whereas convection enhanced delivery is able to affect tissue that is much further from the delivery site.

However, current CED protocols that use standard needles and catheters may not be suitable for chronic applications due to several possible problems: tissue damage from mismatch in mechanical properties between device and brain tissue, backflow along the catheter path, and immune responses. Implantable microfabricated fluidic devices may be able to alleviate these issues due to their small cross-sectional area.

Silicon based microfluidic devices moderate the initial tissue damage due to insertion and backflow problems, but they may still not be suitable for long-term CED therapies. Silicon is a brittle material that may break inside the tissue, and silicon probes have been found to cause a significant immune response [5].

Flexible polymer microfluidic devices demonstrate better biocompatibility than silicon probes but their low rigidity makes them difficult to insert into tissue. In an effort to overcome this problem we made flexible parylene microfluidic devices and constructed rigid scaffolds from poly(DL-lactide-*co*-glycolide) (PLGA). These scaffolds support the flexible parylene device during insertion, but then disappear to leave the flexible fluidic device in place in the tissue.

PLGA is a biodegradable polyester that degrades through hydrolysis of the ester linkages by water that is taken up from the surrounding tissue; this hydrolysis is accompanied by gradual erosion and eventual disappearance of the implanted device. The final degradation products are lactic acid and glycolic acid, which are water soluble, non-toxic products of natural metabolism. PLGA products are widely used in biomedical engineering applications as resorbable sutures [6], microspheres for controlled drug delivery [7], and tissue engineering scaffolds [8].

PLGA structures are generally formed in two ways, through solvent removal techniques (e.g. to produce microspheres [7], [8]) and through melt processing, where the polymer is heated above its glass transition temperature and formed into the appropriate shape (such as tissue engineering scaffolds [10], [11]).

In this work the flexible microfluidic devices were constructed using top-down microfabrication techniques on a silicon wafer and the degradable scaffolds were formed by hot-embossing the PLGA in a poly(dimethysiloxane) (PDMS) mold.

Channels with cross sectional areas of 10 μm x 50 μm were attached to scaffolds and inserted into a 0.6 % w/v agarose gel brain phantom. Also, the *in vitro* degradation time of the scaffolds was determined and

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found to be in accordance with previous reports.

These results show that a biodegradable scaffold is a viable method for easing the insertion of implantable flexible microdevices.

Materials and Methods

The main body of the microfluidic device was constructed using standard micro-machining techniques on a silicon wafer. The manufacturing process is shown in figure 1.

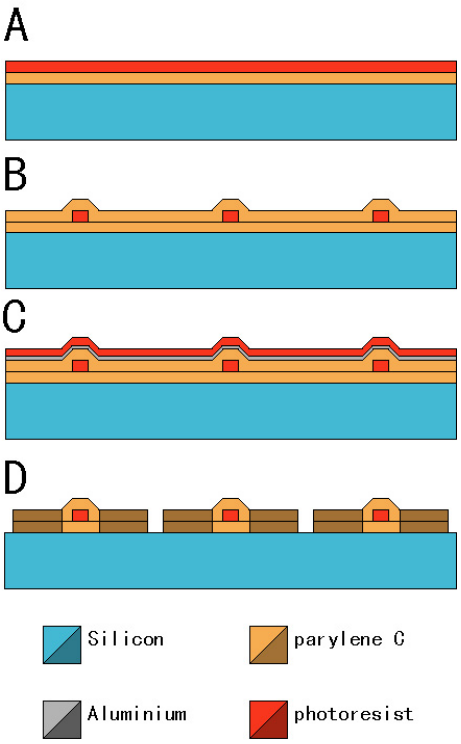

Figure 1: Manufacturing process for microfluidic devices

Stage A involved pre-treating the wafers in a CF_4 plasma to roughen the silicon before a layer of parylene-C was deposited using chemical vapor deposition (PDS 2010 LabCoter, Speciality Coating Systems). This layer formed the base of the fluidic device. Next, photoresist (Shipley 1045 positive tone) was spun on the wafers to a depth of 10 μm (the intended height of the channels). Stage B involved patterning the initial layer of photoresist using contact lithography (EV-620 contact aligner). The remaining photoresist formed the inside of the channels. A second layer of parylene C was then deposited to form the top of the microfluidic device. Stage C involved depositing a 50 nm thick aluminium etch mask via e-beam evaporation (CVC SC4500 E-beam evaporation system). This mask was then patterned with a second photolithography step, and the exposed aluminium was removed in a wet-etch. Stage D required that the photoresist wet-etch mask be stripped by soaking in acetone before the device body could be defined using an oxygen RIE (Oxford PlasmaLab 80+ RIE system) to etch through the parylene to the silicon wafer. Once the ends of the channels had been opened, the resist inside the channels was removed by soaking the wafers in acetone. The probes could then be easily removed from the wafer to form free-standing parylene microfluidic devices.

The PLGA insertion scaffold was made by hot-embossing PLGA granules in a poly(dimethylsiloxane) (PDMS) mold. To form the master for the molding, a silicon wafer was patterned using photolithography and selectively etched using a Bosch process (Unaxis SLR 770) to a depth of 200 μm. This wafer was then silanized with tridecaflouro – 1,2,2 tetrahydrooctyl trichlorosilane before the PDMS mold was cast to prevent the mold from bonding to the master. To cast the PDMS mold, PDMS base and curing agent (Sylgard 184 silicone elastomer kit, Dow Corning) were mixed in a 10:1 weight ratio, and the mixture was thoroughly degassed in a vacuum chamber. The mixture was then poured over the silicon master and cured in an oven at 70 °C for three hours. When the mold had cured, it could be peeled from the master and used for hot-embossing.

The scaffolds were formed using a method similar to that demonstrated by Yang *et al* [10]. Initially, the mold was spin coated with a 17 % wt/v aqueous poly(vinyl alcohol) (PVA) solution (M_w 31,000 – 50,000, Sigma chemical company). Granules of PLGA (5050 DL 3.5A, Lakeshore Biomaterials) were placed on the mold and a second piece of non-patterned PDMS was placed on top. This sandwich was placed in a laboratory press with heated platens, heated to 150 – 160 °C and pressed at approximately 3.5 MPa for 5 – 10 minutes. The assembly was cooled to below the glass transition temperature of the PLGA by circulating water through the platens. The sandwich was then disassembled, and the PVA sacrificial layer was dissolved in water. The PLGA scaffolds could then be cleanly removed from the mold.

To connect the parylene devices to a fluidic circuit they were first removed from the wafer and glued to the end of a 500 μm internal diameter glass pipette (World Precision Instruments Inc.) using two-part epoxy. The pipette was then backfilled with the fluid to be infused (usually an Evan's blue dye) and connected to a programmable pressure injector (PM8000, World Precision Instruments Inc.). To make a fluidic probe insertable a PLGA scaffold was placed against the fluidic probe and held in place using surface tension due to a drop of water. This scaffold/microfluidic assembly was then mounted in a micromanipulator and inserted into a 0.6 wt % agarose gel brain phantom and fluid was infused at pressures ranging from 35 – 200 kPa. The effect of the scaffold on the fluidic channel was investigated by monitoring the flow rate obtained for a range of driving pressures for channels with and without a scaffold attached.

To investigate the degradation time of the PLGA scaffolds, samples were weighed and placed in a 50 mM HEPES buffer containing 10 mM KCl and 0.1 % wt/v

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NaN_3 (pH 7.4) and incubated at 37 °C. Samples were removed every three days, washed, lyophilized overnight and examined for gravimetric weight loss. The pH of the buffer was monitored and replaced with fresh buffer if the acidic degradation products caused the pH to drop below 7.0.

Results

The parylene microfluidic devices had an overall length of 9.075 mm, and had a 2 mm wide body for ease of handling. The insertable shank was 150 μm wide and 3 mm long, and the channel was 10 μm high and 50 μm wide. A parylene probe is shown in figure 2. The flexibility of the microfluidic probe is demonstrated in figure 3.

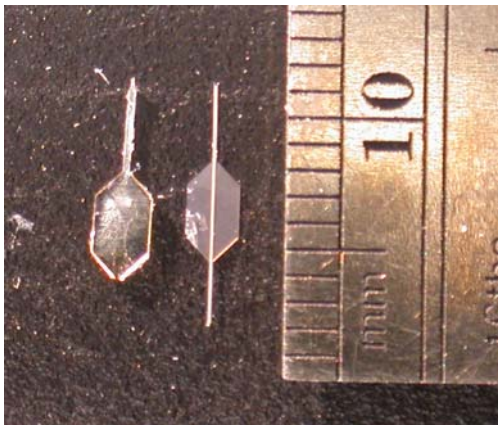

Figure 2: Photograph of PLGA scaffold (left) and flexible fluidic device (right).

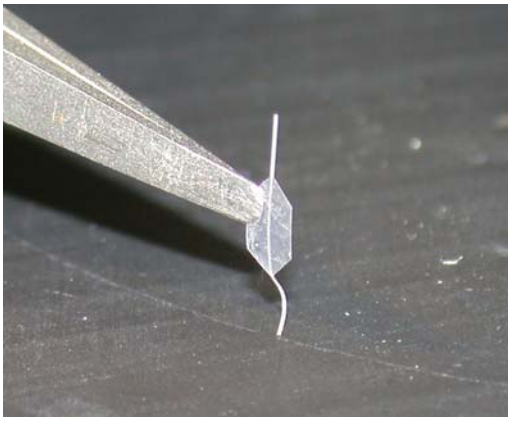

Figure 3: Photograph of the flexible parylene fluidic device.

The hot embossing technique was used to rapidly produce scaffolds with good geometric resolution. The scaffolds were measured under an optical microscope and found to be slightly larger than the original silicon master (< 10%). The scaffolds were designed to have an implantable shank of 150 μm wide by 200 μm tall and of length 3.225 mm. A PLGA scaffold is shown in figure 2, above.

The fluidic devices were found to be easily inserted into agarose gel phantoms, with the surface tension of the water being great enough to hold the fluidic channel in place on the scaffold shank. Also, the addition of the scaffold had no effect on the flow characteristics of the channel and a flowrate of 3.05 μl/min was obtained at a driving pressure of 103.4 kPa for Evan's blue solution. (Figure 4). We were able to infuse Evan's blue solution into the brain phantoms with minimal backflow, and the observed distributions closely approximated the spherical distribution of an ideal point source.

The scaffolds used in the degradation study were found to have an average initial weight of 2.37 ± 0.41 mg. The gravimetric weight loss as a function of time in the degradation buffer is shown in figure 5. It can be seen that the scaffolds are almost 100% degraded after 27 days, but they were observed to have no rigidity after only 15 – 18 days. This was comparable with the manufacturer's reported time (3 – 4 weeks).

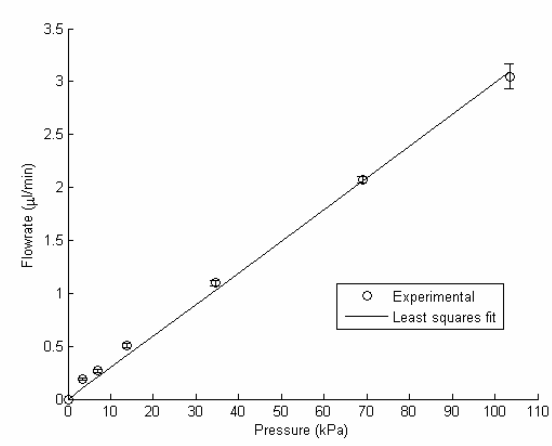

Figure 4: Flow calibration for microfluidic device with attached scaffold using Evan's blue solution. Error bars represent standard deviation of three replicates.

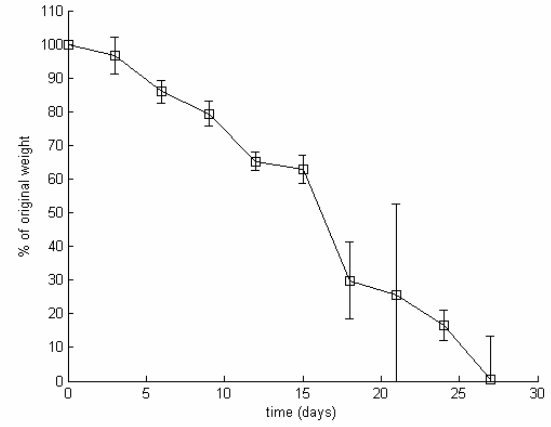

Figure 5: Weight loss as a function of time in degradation buffer. Error bars indicate standard deviation of three replicates.

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Discussion

The results of this study show that it is possible to insert flexible polymeric microfludic devices into agarose brain phantoms and to use these devices to infuse solutions into the gels. Because agarose gels have similar mechanical and transport properties [12] as neural tissue, this finding suggests that this technique could also be useful for chronic CED protocols in the brain,.

The parylene microfluidic devices were capable of delivering relatively large flow rates for CED protocols at modest driving pressures, which is a favorable characteristic for a long-term implantable device as it would reduce the size of the external equipment required. This would make it easier for the device to be made fully portable.

The slight increase in size of the scaffolds when compared with that of the original silicon master is likely due to thermal expansion and pressure induced deformation of the PDMS mold during hot embossing. This effect was also observed by Yang *et al* [10].

Furthermore, PLGA structures have a long history of use in controlled release drug delivery devices [13] and their release characteristics have been well characterized. This means that the insertion scaffolds could be loaded with drugs which could influence the local tissue in order to improve the effectiveness of the CED protocol, e.g. the controlled release of dexamethasone to mediate inflammation [14].

The degradation time of the scaffolds was determined *in vitro* to be about 3 – 4 weeks, which was the same as the manufacturers reported value, and other previous reports. However, Grayson *et al* [15] found that the size of a sample affected degradation time. Larger samples were subject to bulk degradation which accelerated their degradation compared with smaller samples that degraded primarily by surface erosion. In our case the scaffolds were large enough to undergo both surface and bulk degradation and therefore exhibited the reported degradation time.

Also, the degradation time of the scaffolds could be adjusted by changing the molecular weight of the PLGA, by changing the end group, or by changing the lactide to glycolide ratio. This in turn could be used to control the duration of the controlled release which makes this a promisingly variable system.

Conclusions

The results from this study suggest that a biodegradable scaffold can facilitate insertions of implantable polymeric microdevices, without causing any loss in performance. The system developed here also permits versatile design for use in CED protocols as it can employ both diffusive drug delivery (from the scaffold) and convective delivery (from the fluidic channel). Future work on this system will investigate loading the scaffolds with drugs and also examine the *in vivo* performance of the devices in a rat model.

Acknowledgments

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