Material Development and Optimization

Mater. Res. Soc. Symp. Proc. Vol. 1299 © 2011 Materials Research Society
DOI: 10.1557/opl.2011.377

Biodegradable Microfluidic Scaffolds with Tunable Degradation Properties from Amino Alcohol-based Poly(ester amide) Elastomers

Jane Wang[1,2,3], Tatiana Kniazeva[2], Carly F. Campbell[2], Robert Langer[3,4], Jeffrey S. Ustin[5], Jeffrey T. Borenstein[2]

[1]*Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA, 02139*
[2]*Biomedical Engineering, Charles Stark Draper Laboratory, Cambridge, MA, USA, 02139*
[3]*Program of Polymer Science and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA, 02139*
[4]*Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA, 02139*
[5]*Trauma Surgery, Cleveland MetroHealth Hospital, Cleveland, OH, USA, 44106*

ABSTRACT

Biodegradable polymers with high mechanical strength, flexibility and optical transparency, optimal degradation properties and biocompatibility are critical to the success of tissue engineered devices and drug delivery systems. In this work, microfluidic devices have been fabricated from elastomeric scaffolds with tunable degradation properties for applications in tissue engineering and regenerative medicine. Most biodegradable polymers suffer from short half life resulting from rapid and poorly controlled degradation upon implantation, exceedingly high stiffness, and limited compatibility with chemical functionalization. Here we report the first microfluidic devices constructed from a recently developed class of biodegradable elastomeric poly(ester amide)s, poly(1,3-diamino-2-hydroxypropane-co-polyol sebacate)s (APS), showing a much longer and highly tunable in vivo degradation half-life comparing to many other commonly used biodegradable polymers. The device is molded in a similar approach to that reported previously for conventional biodegradable polymers, and the bonded microfluidic channels are shown to be capable of supporting physiologic levels of flow and pressure. The device has been tested for degradation rate and gas permeation properties in order to predict performance in the implantation environment. This device is high resolution and fully biodegradable; the fabrication process is fast, inexpensive, reproducible, and scalable, making it the approach ideal for both rapid prototyping and manufacturing of tissue engineering scaffolds and vasculature and tissue and organ replacements.

INTRODUCTION

One of the principal challenges in tissue engineering is the requirement for a vasculature to support oxygen and nutrient transport within the growing tissue. One avenue for achieving this goal is the formation of a microfluidic network within the tissue engineering scaffold; an initial proof of principle for this concept was demonstrated using nondegradable PDMS as the substrate for endothelialized microfluidic networks.[1] Early demonstrations of biodegradable microfluidic devices capable of supporting microvascular networks were reported by Armani and Liu,[2] King et al.,[3] and Liu and Bhatia.[4] The first of these reports required the insertion of a nondegradable metallic layer for bonding the degradable PLGA films; the latter two were

constructed solely from biodegradable PLGA. These PLGA-based structures suffered from excessive mechanical stiffness and sudden changes in mass and mechanical strength during the resorption process, spurring development of alternative biodegradable materials with more optimal mechanical and resorption properties. The first of these was a biodegradable elastomer poly(glycerol sebacate) (PGS).[5] The relatively short half-life of PGS led to further development of APS as a tunable biodegradable elastomer for tissue engineering applications; here we present the first report of a microfluidic network capable of serving as an intrinsic vasculature formed from the APS polymer.

To develop a longer-lasting scaffold substrate than PGS while preserving its excellent chemical and mechanical properties, a new class of biodegradable elastomers, poly(ester amide), poly(1,3-diamino-2-hydroxypropane-co-polyol sebacate) (APS) with tunable degradation rates was developed.[6] This class of APS materials encompasses a wide range of chemical compositions. These compositions have been shown to be highly biocompatible, with primary hepatocyte culture exhibiting cell functionality over extended periods without the need for deposition of protein coatings on the surface. In addition, the APS surface is amenable to nanostructuring to provide topographic features representative of the cell microenvironment.[7] In this work, we present microfabrication results for microfluidic channel networks as well as in vitro degradation data as a function of selective enzyme triggers for two APS polymer compositions. These data demonstrate that the APS system represents a novel class of polymers for the development of microfluidic scaffolds for tissue engineering applications.

EXPERIMENTAL METHODS

Synthesis of poly(1,3-diamino-2-hydroxypropane-co-glycerol sebacate) elsatomers

All materials were purchased from Sigma Aldrich (St. Louis, MO, USA) and used as received unless otherwise specified. A round bottom flask was charged with 0.06 mol of 1,3-diamino-2-hydroxy-propane (DAHP), 0.03 mol glycerol (G), and 0.09 mol of sebacic acid (SA) to produce a molar ratio of 2:1:3 of DAP:G:SA, respectively to produce 2-1 APS, while 1-2 APS was synthesized with a molar ratio of 1:2:3. The reactants were heated under an argon blanket at 130 ^{o}C for 3 h. The pressure was then dropped to approximately 50 mTorr and the contents were allowed to react for 10 h at 130 ^{o}C. The product was then stored under a desiccant environment until further use.

Microfabrication of microfluidic scaffold using reverse silicon molding

The silicon mold was designed and created as previously described by King *et al.*[3] Briefly, prior to replica molding of APS, a sacrificial maltose release layer was spin-coated on the silicon master. Photolithographically patterned silicon masters were cleaned using piranha solution (Mallinckrodt, St. Louis, MO) and oxygen plasma-cleaned (March, St. Petersburg, FL) at 250 mTorr and 200 W for 45 seconds. A 60% (w/w) solution of maltose (Sigma, St. Louis, MO) in water was spin-coated at 2500 revolutions per minute for 30 s. The maltose layer was pre-baked on a hot plate at 95 °C for 120 seconds. 5.00 ± 0.05 g of APS prepolymer was melted at 170 °C and applied to the wafers for replica molding and smooth sheet formation. The prepolymer was cured at 170 °C for 48 h under 50 mTorr of vacuum, which produced a crosslinked sheet in which a portion of the hydroxyl and carboxylic acid functional groups

remained. The APS sheets were delaminated by statically incubating the polymer-master system in doubly distilled water (ddH2O) at 60 °C for 24 h beginning immediately after polymer curing. Diffusion of water between the polymer/silicon interfaces led to maltose dissolution and eventual delamination. Sheets were trimmed and punched to achieve appropriate fluidic connections between layers. Microfluidic APS layers were stacked, aligned, and bonded together simultaneously by oxygen plasma treatment for surface activation, followed by curing the polymer at 170 °C for 24 h under 50 mTorr of vacuum. An additional layer of PDMS was laminated onto the device for tubing stabilization. Once the final curing step was completed, silicone tubing (1/16 in. inner diameter, 1/8 in. outer diameter, Cole-Parmer) was inserted into the devices in a sterile environment. Luer-Lok connections were inserted into the tubing, and the base of the connections was sealed with epoxy (McMaster-Carr).

Microfabrication of microfluidic scaffold using reverse PDMS molding

The SU-8 patterned wafer was designed and created as previously described by Bettinger et al.[8] Prior to replica molding of PDMS, fluoropolymer was coated as passivation layer on the master. 25.00 ± 0.05 g of PDMS prepolymer was applied to the wafers for PDMS replica molding. The PDMS was crosslinked at 70°C for 4 h. Prior to replica molding of APS on PDMS, similar maltose coating procedures to the previous section were employed. 5.00 ± 0.05 g of APS prepolymer was melted at 170 °C and applied to the wafers for replica molding and smooth sheet formation. The prepolymer was cured at 170 °C for 48 h under 50 mTorr of vacuum, which produced a crosslinked sheet in which a portion of the hydroxyl and carboxylic acid functional groups remained. The APS sheets were delaminated by statically incubating the polymer-master system in doubly distilled water (ddH2O) at 60 °C for 24 h beginning immediately after polymer curing. Diffusion of water between the polymer/PDMS interfaces led to maltose dissolution and eventual delamination. Devices were assembled using similar techniques as described in the previous section.

Degradation of 2-1 APS and 1-2 APS

Cylindrical polymer slabs (n=3) with dimensions of 0.5mm × 6mm (T × D) and weighing ~20mg each were incubated at 37°C in the following degradation media: (1) Dulbecco's Phosphate Buffer Saline (DPBS); (2) 10 U mL^{-1} of protease I from bovine pancreas in DPBS; (3) 10 U mL^{-1} lipase II from porcine pancreas in DPBS. Buffer and enzyme solutions were exchanged every 2 days and dry mass loss measurements were made at specific time points. Samples were washed in ddH_2O, incubated in ethanol and ddH_2O for at least 24 h. Dry mass were weighed for mass loss comparison.

DISCUSSION

Microfabrication of microfluidic scaffold

The basic technical approach for designing and constructing a microvascularized scaffold device here is similar to that reported for PLGA,[3] PGS[8-9] and silk fibroin.[10-11] Briefly, a simple microchannel network design was generated based on principles of microvascular flow, pressure and wall shear stress.[12] This microchannel network is based upon design principles

including uniform flow and distribution of oxygen and nutrients throughout a scaffold layer, as well as physiologic levels of pressure drop and wall shear stress within the various vessel sizes in the network. The network was translated into a photolithographic layout for fabrication, with the intent of producing a single-layer microvascular network.

For PGS, the optimal surface coating was found to be sucrose dissolved in water, but process development with APS determined that the temperatures involved in curing resulted in unacceptably high levels of sucrose caramelization during the process. Therefore, we investigated alternative sacrificial sugar coatings and determined that maltose has a caramelization temperature about 20 ^{o}C higher than sucrose. Therefore, maltose coatings were deposited onto the silicon masters to assist in delamination of APS films, and APS was cast onto the silicon master molds and then lifted off as a free-standing film. A channel layer was bonded to a flat APS layer and the layers joined at elevated temperature and pressure. The challenge for layer bonding is to form a strong, irreversible and leakproof bond between films, without raising the temperature or pressure so high as to deform or collapse the microchannels or other high resolution structures. In order to prepare the bonded devices for future flow testing and cell seeding, silicone tubing was attached to the APS microfluidic scaffold to demonstrate flow through the network at physiological pressures (0-180 mmHg). As seen in Figure 1, robust microfluidic devices were built using APS, and exhibited good flow behavior. It was shown that unlike softer alternative materials, the strength of APS was sufficient to support maintenance of the channel height, as well as suitable flow dynamics.

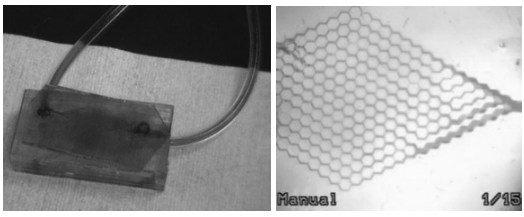

Figure 1. a) Full microfluidic channel device in APS flowing with red dye. b) Microfluidic channels mimicking blood vessel bifurcated networks built in APS flowing with red dye.

Degradation of 2-1 APS and 1-2 APS

The comparison of degradation properties between 2-1 APS and 1-2 APS showed that by varying the ratio of diamine to glycerol (2:1 for 2-1 APS and 1:2 for 1-2 APS) the degradation rate of APS is tunable as shown in Figure 2. The higher the diamine content, the slower the polymer degrades, and the less the polymer responds to lipase solution as a catalyst for degradation.

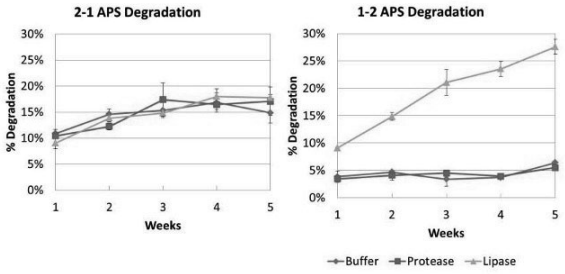

Figure 2. a) 2-1 APS degradation over 5 weeks in buffer, protease, and lipase solution. B) 1-2 APS degradation over 5 weeks in buffer, protease, and lipase solution.

It is also shown in Figure 3 that the polymer undergoes surface erosion as oppose to bulk resorption, which is beneficial in maintaining functionality of the device during degradation.

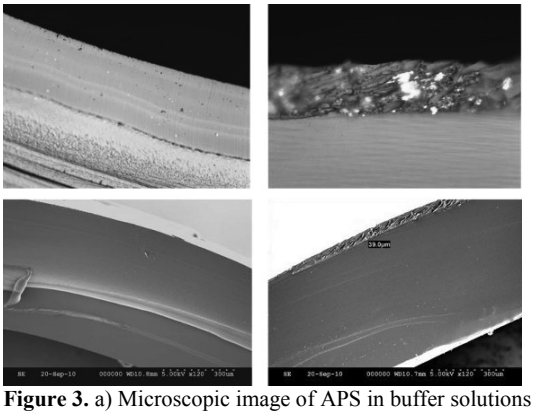

Figure 3. a) Microscopic image of APS in buffer solutions for 4 days. b) Microscopic image of APS in lipase solution for 4 days. c) SEM of APS in buffer solution for 4 days. d) SEM of APS in lipase solution for 4 days.

CONCLUSIONS

The concept of biodegradable microfluidic devices was first introduced using PLGA in by Armani and Liu[2] and King et al.,[3] but these device constructs exhibit a high degradation rate, low elasticity, and concerns regarding immune and inflammatory response in bulk format. More recently, Fidkowski *et al.*[9] and Bettinger *et al.*[8] constructed PGS-based devices that exhibit much more desirable mechanical properties for implantation, but the relatively high degradation rate of the polymer limits its applicability for certain long-lasting applications as an implant. In the present study, we report on the development of a specific process for biodegradable microfluidic devices constructed from APS, an alcohol-based poly(ester amide) elastomer, which possesses a much lower degradation rate but retains elastomeric properties for tissue scaffold applications. The process reported here is capable of forming microfluidic channels on a wafer-

scale with strong layer-to-layer bonds and well-preserved microscale architecture. Robust flow conditions within the devices were developed. The polymer also showed selective degradation toward lipase solution. It was also observed that by varying the amine to glycerol ratio, the degradation properties of APS can be tuned. The biocompatibility of APS *in vivo* by Bettinger et al.[13] demonstrated that the material could be used for resorbable tissue engineering devices for the purpose of drug delivery and regenerative medicine. The next steps for this investigation will focus on the seeding of endothelial cells to form a functional microvascular network, and implantation studies to explore in vivo biocompatibility, host integration and long-term degradation properties for this scaffolding material.

ACKNOWLEDGMENTS

Funding for this work, provided by Draper Laboratory and by the American Association for the Surgery of Trauma, is gratefully acknowledged. We are indebted to G.C. Engelmayr, C.J. Bettinger, L.E. Freed, J. Hsiao, and E. Kim for many useful discussions and assistance with the instrumentation and polymer synthesis.

REFERENCES

1. J. T. Borenstein, H. Terai, K. R. King, E. J. Weinberg, M. R. Kaazempur-Mofrad and J. P. Vacanti, Biomedical Microdevices **4** (3), 167-175 (2002).
2. D. K. Armani and C. Liu, Journal of Micromechanics and Microengineering **10** (1), 80-84 (2000).
3. K. R. King, C. C. J. Wang, M. R. Kaazempur-Mofrad, J. P. Vacanti and J. T. Borenstein, Advanced Materials **16** (22), 2007-12 (2004).
4. V. Liu Tsang, A. A. Chen, L. M. Cho, K. D. Jadin, R. L. Sah, S. DeLong, J. L. West and S. N. Bhatia, FASEB J **21** (3), 790-801 (2007).
5. Y. D. Wang, G. A. Ameer, B. J. Sheppard and R. Langer, Nature Biotechnology **20** (6), 602-606 (2002).
6. C. J. Bettinger, J. P. Bruggeman, J. T. Borenstein and R. S. Langer, Biomaterials **29** (15), 2315-2325 (2008).
7. C. J. Bettinger, Pure and Applied Chemistry **81** (12), 2183-2201 (2009).
8. C. J. Bettinger, E. J. Weinberg, K. M. Kulig, J. P. Vacanti, Y. D. Wang, J. T. Borenstein and R. Langer, Advanced Materials **18** (2), 165-9 (2006).
9. C. Fidkowski, M. R. Kaazempur-Mofrad, J. Borenstein, J. P. Vacanti, R. Langer and Y. D. Wang, Tissue Engineering **11** (1-2), 302-309 (2005).
10. J. T. Borenstein, M. M. Tupper, P. J. Mack, E. J. Weinberg, A. S. Khalil, J. Hsiao and G. Garcia-Cardena, Biomedical Microdevices **12** (1), 71-79 (2010).
11. C. J. Bettinger, K. M. Cyr, A. Matsumoto, R. Langer, J. T. Borenstein and D. L. Kaplan, Advanced Materials **19** (19), 2847-50 (2007).
12. J. T. Borenstein, E. J. Weinberg, J. P. Vacanti and M. R. Kaazempur-Mofrad, in *Micro and Nanoengineering of the Cell Microenvironment*, edited by A. Khademhosseini, J. T. Borenstein, M. Toner and S. Takayama (Artech House, Boston, 2008).
13. C. J. Bettinger, K. M. Kulig, J. P. Vacanti, R. Langer and J. T. Borenstein, Tissue Engineering Part A **15** (6), 1321-1329 (2009).

Mater. Res. Soc. Symp. Proc. Vol. 1299 © 2011 Materials Research Society
DOI: 10.1557/opl.2011.63

Measurements of Resonance Frequency of Parylene Microspring Arrays Using Atomic Force Microscopy

C. Gaire[1], M. He[1], A. Zandiatashbar[2], P.-I. Wang[1], R. C. Picu[2], G.-C. Wang[1] and T.-M. Lu[1]
[1]Department of Physics, Applied Physics and Astronomy, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY, 12180, U. S. A.
[2]Department of Mechanical, Aeronautical and Nuclear Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY, 12180, U. S. A.

ABSTRACT

A mechanical vibration system was made by sandwiching an array of parylene-C microsprings between two flat plates of Si. This system was driven mechanically in forced oscillation using a piezo transducer attached to the bottom Si plate. An atomic force microscope was used to record the displacement of the top plate in both the contact and non-contact modes. At the resonance, the system was observed to give large vertical displacement amplitude of up to 100 nm with a Q-factor of up to 900.

INTRODUCTION

Recent advents of micro- or nanospring arrays for optical interferometry [1], pressure sensing [2] and electromechanical actuation [3,4] have generated a significant interest in the use of such structures as elements of micro- and nanoelectromechanical systems (MEMS and NEMS). Most of the current MEMS or NEMS elements are made of Si microstructures that inherit the advantages of fabrication by planar processing techniques used in silicon microelectronic technology [5-7]. The use of microstructures made of polymers such as parylene as elements of MEMS or NEMS is less developed to date [8,9]. Due to its low elastic modulus, high structural flexibility, chemical robustness and ease of fabrication, parylene microstructures could play an important role in future MEMS or NEMS devices. The ability of an accurate measurement of natural frequency of such structures is required to gain control over the desired precision in the mechanical motion. Hence, resonance frequency measurement is one of the most important prerequisite of MEMS or NEMS elements.

In this article, we present the results of resonance frequency measurement of a mechanical vibration system composed of an array of parylene-C microsprings using an atomic force microscope (AFM). The system contains several millions microsprings sandwiched between two Si plates. A piezoelectric transducer is used to drive the system from the bottom plate and the AFM is used to record the displacement of the top plate.

EXPERIMENT

Parylene spring growth

The growth of parylene-C microsprings was carried out by oblique angle deposition [10]. We have employed a substrate swing rotation scheme during deposition to control the size and uniformity of the springs [11,12]. In short, the parylene vapor sublimated at 190 ^{o}C was passed

through a pyrolysis furnace at 680 ^{o}C to convert to parylene-C monomers. This monomer flux was guided by a nozzle [10] and was deposited on Si(001) wafer at an angle of ~ 85^{o} with respect to the substrate normal. The parylene-C monomers polymerize upon deposition onto the substrate [13]. Due to the shadowing effect and limited mobility of deposited material, isolated islands are formed on the surface and serve as seeds for the growth of microsprings. The substrate was rotated in swing motion at 10 rpm within the azimuthal angle of 90^{o} without changing the deposition angle with respect to the substrate normal. Each turn of the spring consists of four discrete units grown by successive deposition of arms with substrate turned by 90^{o} after the completion of each arm. These springs had the following dimensions: wire diameter, t = (2.3±0.6) μm; spring pitch, h = (7.5±0.3) μm, coil diameter D = (5.0±0.5) μm, rise angle of ~ 30^{o}, and number of turns, n = 4.

Mechanical vibration system

The mechanical vibration system was made by bonding the sample to a Si piece (dimensions: 12.5 mm × 12.7 mm × 725 μm; mass: 268 mg) through ~2 μm thick epoxy siloxane polymer (obtained from Polyset Co. Inc.) [14] spin-coated on one side and pressed from the other side with a force of 2 N (pressure =12.6 kN/m^{2}). The bonded sample is annealed in inert gas environment at 160°C for one hour to remove the residual solvent inside the epoxy siloxane polymer film and harden this adhesive layer. The lower plate is glued to a piezoceramic sheet and the upper plate is free to oscillate in unison to the microsprings grown on the bottom plate. The piezoceramic sheet was 191 μm thick lead zirconate titanate (PZT) with nickel electrodes (Piezo Systems Inc., Cambridge, MA). The normal strain coefficient of the sheet was 390 pm/V. The PZT sheet was sinusoidally driven with peak-to-peak voltage of 1.0–7.0 V (peak-to-peak amplitude: 0.39-2.73 nm) in two frequency ranges: 0.001–0.1 MHz (using SR830 Lock-in amplifier, Stanford Research Systems, Sunnyvale, CA) and 0.1–100 MHz (using HP 8656B function generator).

Spring constant determination

The stiffness of an individual spring was determined using nanoindentation (NanoTest, MML, Wrexham, UK) on parylene sample without the top Si plate. A flat punch nanoindentation tip of diameter 50 μm was used to indent on 24 different locations. Based on the pitch of the spring array, diameter of the spring wire and the size of the indenter tip, it was estimated that this tip indented 80±10 springs in each indentation. The individual spring constant is determined to be k =21±5 N/m by dividing the total stiffness by the number of springs loaded in each location. The loading-unloading curves were not perfectly overlapping to each other, so the stiffness was determined from the unloading portion of the nanoindentation curves. This spring constant will be used to estimate the theoretical resonance frequency of the vibrating system.

Resonance frequency measurement

An XE-100 Series AFM (PSIA Inc., Santa Clara, CA) was used to measure the vertical amplitude of the top plate while the actuation frequency is swept from 1 kHz to 100 MHz. The displacement of the top plate was measured in both non-contact and contact modes of the AFM. In non-contact mode, the displacement of the top plate was measured dynamically by vibrating