A biphasic elastomeric scaffold for tissue engineering a small-diameter blood vessel

Jian Yang, Delara Motlagh, Antonio R. Webb, Guillermo A. Ameer
Northwestern University, Biomedical Engineering Department, Evanston IL 60208

Introduction

Scaffolds used for tissue engineering should mimic the native structure of target tissues, gradually degrade with tissue formation, and coexist in a mechanically dynamic environment (sustain and recover from various deformations without mechanical irritations to the surrounding tissues[1, 2]). In the anatomy of a native blood vessel, endothelial cells are separated from surrounding smooth muscle cells (SMC) by the internal elastic lamina. To mimic this fact, we have fabricated a biphasic scaffold that recapitulates such cell compartmentalization in a tissue engineered construct. Herein, a newly developed biodegradable elastomeric polyester, poly(diol-co-citrate) is investigated for use as a scaffold material for this design. The biphasic scaffold consists of a solid, continuous phase enabling the formation of a monolayer of endothelia cells (EC) within the vessel lumen and a surrounding porous phase with inter-connective pore structure for SMC growth and differentiation. The scaffold was subjected to various characterization, including burst pressure as well as co-culture with SMC and EC. Cells attached, proliferated and were compartmentalized within the biphasic scaffold.

Experimental

Preparation of poly(1,8-Octanediol-co-citric acid) (POC)[3]: All chemicals were purchased from Sigma-Aldrich (Milwaukee, WI). Equimolar amounts of citric acid and 1,8-octanediol were added to a 250 ml three-neck round-bottom flask, fitted with an inlet and outlet adapter. The mixture was melted under a flow of nitrogen gas by stirring at 160˚C -165˚C in a silicon oil bath, and then the temperature of the system was lowered to 140˚C. The mixture was stirred for another hour at 140˚C to create the pre-polymer solution. The pre-polymer was post-polymerized at 37˚C, 60˚C, 80˚C, or 120˚C under vacuum (2 Pa) or no vacuum for times ranging from 1 day to 2 weeks to create POC with various degrees of cross-linking. Several citric acid based elastomers were synthesized as described above using other diols. The resulting copolymers were poly(1,6-hexanediol-co-citric acid) (PHC), poly(1,10-decanediol-co-citric acid) (PDC), and poly(1,12-dodecanediol-co-citric acid) (PDDC). Polyethylene oxide (PEO) with different molecular weight and N-methyldiethanoamine (MDEA) were also used as third monomer to implement the polycondensation to prepare poly(1,8-octanediol-co-citric acid-polyethylene oxide) (POCPEO), poly(1,12-dodecanediol-co-citric acid-polyethylene oxide) (PDDCPEO), poly(1,8-octanediol-co-citric acid-methyldiethanoamine) (POCM) and so on.

Biphasic scaoffod fabrication: Biphasic scaffolds consist of outside porous phase and inside non-porous phase. The non-porous phase is expected to provide a continuous surface for EC adhesion and spreading, mechanical strength, and elasticity to the scaffold. The porous phase will faciliate the 3-D growth of smooth muscle cells. Biphasic scaffold were fabricated via following procedures. Briefly, glass rods (~3 mm diameter) were coated with the pre-polymer solution and air dried to allow for solvent evaporation. Wall thickness of the tubes were controlled by the number of coatings and the percent pre-polymer in the solution. The

pre-coated pre-polymer was partially post-polymerized under 60°C for 24 hr, then the pre-polymer-coated glass rod is then inserted concentrically in a tubular mold that contains a salt/pre-polymer slurry. The pre-polymer/outer-mold/glass rod system is then placed in a oven for further post-polymerization. After salt-leaching[4], the biphasic scaffold was then de-molded from the glass rod and freeze dried. The resulting biphasic scaffold was stored in a desiccator before use.

Mechanical tests for porous scaffold: Mechanical properties of cylindrical porous scaffolds (6 mm height and 6 mm in diameter) were examined by using an Instron 5544 mechanical tester at a crosshead speed of 2 mm min-1 and a 10 N load cell. To examine the elastic recovery properties, the samples were compressed to one fifty of its original length. After removing the compressive force, the length recovery of cylindrical samples were measured. The compressive recovery ratio was calculated by using Eq. (1) POC tube burst pressure measurement: Burst pressure testing was performed by inflating POC and PDDC tubes (3.65 mm inner diameter, 0.15 mm wall thickness) and recording the pressure at failure. A syringe pump (Sage Instruments, Boston, MA) with one 60 ml syringe was connected with Tygon tubing to one end of the POC tube while the other end was affixed with tubing to a pressure gauge (Cole Palmer, Vernon Hills, IL). The syringe pump was programmed to pump PBS at a rate of 0.67 ml/min through the tube while pressure was measured. Burst pressure was defined as the highest pressure value attained prior to failure.

$$\text{Recovery}(\%) = \frac{L_1}{L_0} \times 100 \qquad (1)$$

Co-culture of human smooth muscle cells (HASMC) and human endothelial cells (HAEC) on biphasic scaffold: HASMC and HAEC were co-cultured on POC biphasic scaffold (Both outside and inside phase are POC) post-polymerized under 80ºC, no vacuum, 2 days using culture medium consisting of three part SmGM-2 and one part EBM-2 (Clonetics, Walkersville, MD). First, HASMC with a density of 9.0×10^6/ml were seeded into the porous phase of both end-blocked biphasic scaffold. One day after HASMC seeding, one end of the scaffold was cut open and the other end remained blocked and HAEC (2.6×10^5/ml) were seeded into the inside lumen of the scaffold. The SMC and EC-seeded scaffold were put into a 6-well culture plate and stayed in incubator for half an hour and then 6 ml co-culture medium was added. Medium was changed every two days. The cell seeded construct was subjected to SEM observation and H&E staining.

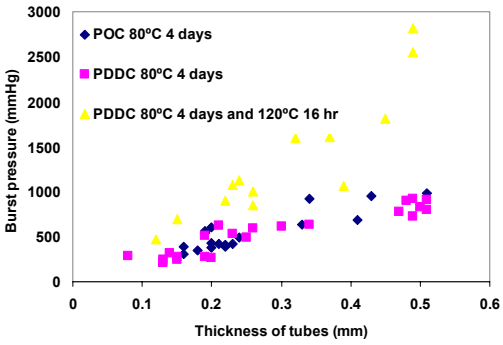
Results and Discussions

Novel biphasic scaffolds were fabricated successfully by using a family of citric acid–based biodegradable elastomers. The mechanical tests of porous POC scaffolds show that the scaffolds are soft (low modulus) and elastic (almost complete recovery, elongation can be over 260% and 400% in the case of POC and PDDC films respectively). The thickness, degradation, and mechanical properties of inside non-porous phase can be well controlled by choosing various pre-polymers of this family of elastomers, pre-polymer concentration, coating times and post-polymerization conditions (burst pressure can be as high as 2800 mmHg). The degradable porous phase and non-porous phases are integrated since they are formed in-situ via post-polymerization. The cell culture experiments confirm that both HAEC and HASMC can

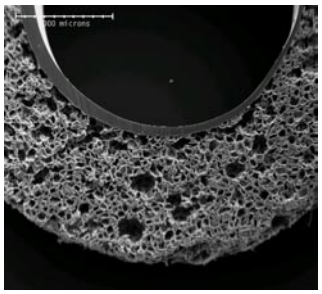
attach and grow well in biphasic scaffolds. The results suggest that a biphasic scaffold design based on poly(diol-co-citrate) is a viable strategy towards the engineering of small diameter blood vessels.

Table 1 Mechanical tests and recovery of porous POC scaffold

Porous size of POC scaffold (μm)	Compressive strength at 10 N load cell (MPa)	Compressive modulus (MPa)	Compressive strain at 10 N load cell (%)	Recovery (%)
90±16	0.437±0.023	0.152±0.018	62.0±4.0	99.9%
41±10	0.449±0.029	0.178±0.052	63.2±3.7	



A)



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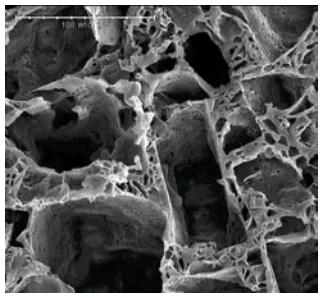

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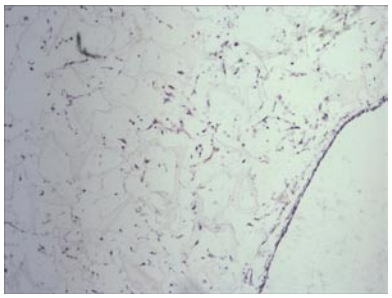

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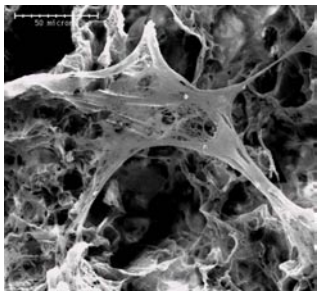

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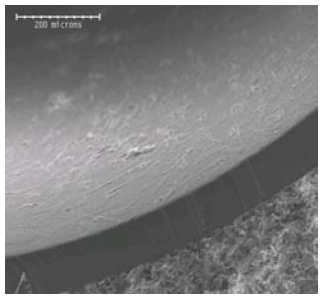

F)

Figure 1 A) Burst pressure of POC and PDDC polymerized under different conditions with different thickness; B) SEM picture of cross section of POC biphasic scaffold; C) SEM picture of pore structure of porous phase; D) H&E staining of SMC seeded POC scaffold for 1 week; E) HASMC on porous phase of co-cultured biphasic scaffold; F) HAEC on lumen of co-cultured biphasic scaffold;

References

1. M.P. Lutolf, G.P. Raeber, A.H. Zisch, N. Tirelli, and J.A. Hubbell. Adv. Mater., 2003. **15**(11): p. 888-892.
2. Y. D. Wang, G. A. Ameer, B. J. Sheppard, and R. Langer. Nature Biotechnology, 2002. **20**: p. 602-606.
3. J. Yang, A.R. Webb, and G.A. Ameer. Adv. Mater. 2004. **16**(6): p. 511-516.
4. J. Yang, G. X. Shi, J. Z. Bei, S. G. Wang, Y. L. Cao, Q. X. Shang, G. H. Yang, and W. J. Wang. J. Biomed. Mater. Res., 2002. **62**(3): p. 438-446.