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Fabrication and Characterization of Tough Elastomeric Fibrous Scaffolds for Tissue Engineering Applications

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Abstract

Development of biodegradable tough elastomeric scaffolds are important for engineering tissues such as myocardium and heart valves that experience dynamic environments *in vivo*. Biomaterial scaffolds should ideally provide appropriate physical, chemical and mechanical cues to the seeded cells to closely mimic the native ECM. Collagen fibers form an important component of native myocardium as well as heart valve leaflets and provide necessary tensile properties to these tissues. Amongst various polymers, collagen mimicking biodegradable elastomer, Poly-(glycerol-sebacate) (PGS) has shown great promise in microfabricated scaffolds for cardiac tissue engineering. However, its use is limited by its solubility and the ability to cast nano-/microfibrous structures. For its superior mechanical properties, thermal or UV crosslinking of the pre-polymer is required under high temperatures and vacuum limiting fabrication of fibers. In this work, electrospun PGS fibers were fabricated by simply blending it with biodegradable polycaprolactone (PCL) polymer without any post-processing. It was hypothesized that microfibrous PGS-PCL scaffolds would provide appropriate physical (fibrous structure) and chemical (balanced hydrophilicity and hydrophobicity) to the cells in addition to the mechanical properties.

I. INTRODUCTION

The field of tissue engineering or regenerative medicine holds great promise as a means to compensate for donor shortages by creating functional human tissue equivalents. To be successful, this approach has to closely recapitulate the *in vivo* cellular and tissular complexities in terms of physical, chemical and mechanical properties. It is well known that tissue morphogenesis, function and regeneration are closely controlled by cell-matrix interaction^{1, 2}. In fact, the extracellular matrix (ECM) provides the mechanical support as well as spatiotemporally regulated biochemical signals to the cells affecting their proliferation, differentiation, migration and apoptosis. This has led the researchers to synthesize new biomaterials or modify the existing ones to meet these unmet demands. This is especially very important for engineering cardiovascular tissues that experience very dynamic mechanical environment and the area of biodegradable elastomeric polymers is expanding fast to meet these needs³⁻⁵.

Poly(glycerol-sebacate) (PGS) is a biocompatible and biodegradable elastomeric polymer³ that has emerged as a promising scaffold material for tissue engineering applications⁶⁻¹⁴. PGS can be easily synthesized from glycerol and sebacic acid, which have been previously approved by FDA³. Biocompatibility studies of PGS^{3, 15, 16} have shown improved cellular response and hemocompatibility both *in vitro* and *in vivo*. However, despite encouraging results, PGS processability into nano-/microfibers still remains a challenge. Fibrous scaffolds are considered advantageous for tissue engineering due to their extracellular matrix (ECM) mimicking properties, high surface area to volume ratio and anisotropic mechanical properties¹⁷⁻²¹. Also, the contact guidance provided by these fibers can be used to regulate cell orientation and migration^{19, 22-24}. Thus, the ability to fabricate PGS fibers may be beneficial for cardiovascular tissue engineering applications by simultaneously providing contact guidance and superior mechanical properties. However, it is challenging to cast

fibrous PGS scaffolds because PGS pre-polymer has to be processed by thermal curing at high temperature and vacuum to convert it into a tough elastomer^{25–27}. PGS prepolymer itself has low solution viscosity and hence, cannot be electrospun to form stable fibers. Recently, PGS was casted into fibers, however, both these methods required either thermal²⁷ and photocrosslinking^{25, 28}. In this work, we report the fabrication of porous microfibrillar scaffolds of PGS by simply blending it with FDA approved biodegradable polycaprolactone (PCL) with improved mechanical and biological properties.

II. MATERIALS AND METHODS

A. Fabrication and characterization of electrospun scaffolds

PGS:PCL fibers with different compositions were spun by using conventional electrospinning setup. Effects of varying PGS:PCL ratio and voltage on fiber morphology and fiber diameter was studied. PGS and PCL were dissolved at different weight ratios (5:1, 3:1, 2:1 and 0:1, respectively) in anhydrous chloroform: ethanol mixture (9:1) and electrospun at 12.5, 15, 17.5 and 20 kV. The total polymer concentration was kept constant at 33% w/v. Pure PCL scaffolds were electrospun using 16 % w/v polymer solution because it was difficult to electrospin highly viscous 33% w/v PCL solution.

Fibers were characterized for their morphology by using field emission scanning electron microscopy (FE SEM) ultra 55 (Carl Zeiss, Inc. NY, USA). Fiber diameter measurements were performed on at least three different SEM images with 20 fibers per image using ImageJ software NIH, <http://rsbweb.nih.gov/ij/>.

Mechanical properties of the scaffolds were studied using uniaxial elongation test (Instron 5542 mechanical tester, Norwood, MA, USA) on electrospun rectangular meshes ($5 \times 15 \text{ mm}^2$). During uniaxial test, initial strain rate was set as 25% of original test regional length and the crosshead speed was set constant at 2.5 mm/ min. Five specimens from different batches were tested for each condition. Elastic moduli (EM) were calculated from the 0–5% strain region in the stress-strain curve. Ultimate strength (UTS) and ultimate elongation (UE) were measured from the highest peak of stress-strain curve. Three data from each condition were selected from the test results. Values were reported as mean $\pm$ standard deviation (SD).

B. Cell culture

GFP transfected HUVECs were a kind gift from the laboratory of Dr. Judah Folkman, Children's Hospital, Boston. HUVECs were cultured in EBM-2 (Endothelial Cell Basal Medium-2, Lonza, MD, USA) supplemented with the provided growth factor kit. The cell cultures were maintained at 37 °C and 5% CO₂ and media was changed twice a week.

Cells were seeded on each scaffold $\{0.5 \text{ cm (l)} \times 0.5 \text{ cm (w)} \times 200 \mu\text{m (t)}\}$ after sterilization in 70% ethanol at final concentration of 1×10^5 cells/scaffold in 24-well tissue culture plates (TCP) and allowed to attach for 6 h. the scaffolds were then washed three times with DPBS to remove loosely attached/unattached cells. Cells were then fixed with 4% formaldehyde, and washed with DPBS. Cell attachment and cell spreading were observed using FE-SEM, Ultra 55 after palladium-platinum coating.

Quantitative evaluation of cell attachment (after 8 h) and proliferation was performed using Alamar Blue (AB) assay as per manufacturer's instructions (Invitrogen, CA, USA).

C. Statistical analysis

All data were expressed as mean $\pm$ SD. Statistical comparisons between two groups were done using student's paired t-test while multiple comparisons were done using one-way/two-way ANOVA followed by post-hoc Tukey test (OriginPro8 SRO, v8.07). Differences at p-value less than 0.05 were considered to be statistically significant unless otherwise noted. All error bars were presented as standard deviations.

III. RESULTS AND DISCUSSION

Figure 1 shows the SEM images of fibers prepared with different PGS: PCL ratio. Fiber formation showed a very random trend dependent on both PGS concentration as well as applied voltage. The degree of fiber alignment, morphology and diameter was also affected by PGS: PCL ratio as well as the applied voltage. At the same PGS: PCL ratio, increased voltage resulted in reduced fiber diameter due to stretching of polymer jet. Further increase to 20 kV led to fiber fusion and increased fiber diameter (Data not shown). This may be attributed to the jet instability leading to the fusion of fibers. Similarly, increase in PGS amount resulted in increased fiber diameter from $2.76 \pm 0.03 \mu\text{m}$ for PCL to $6.77 \pm 0.26 \mu\text{m}$ for 5:1 PGS: PCL scaffolds (Table 1). This was attributed to lower solution viscosity and waxy nature of PGS pre-polymer.

Mechanical properties (Table 2) showed higher elastic modulus (EM) and ultimate tensile strength (UTS) for 5:1 PGS: PCL scaffolds. Interestingly, increase in the modulus did not compromise on the ultimate elongation (UE) of the scaffold, suggesting its improved toughness while retaining its elasticity. Also, PGS: PCL scaffolds showed mechanical properties comparable to native human aortic heart valve leaflet²⁹, suggesting its potential application in heart valve tissue engineering. It should be noted that such improved mechanical properties could be achieved without any further thermal or UV crosslinking unlike other methods. Fabricated scaffolds were stable with thickness ranging from approximately 100–400 μm .

HUVECs seeded on the PGS scaffolds showed significantly improved attachment and spreading (Fig. 2A) whereas cells remained rounded on PCL-only scaffolds. Addition of PGS also resulted in significantly higher cell proliferation (Fig. 2B) compared to PCL scaffolds ($p < 0.05$). This was attributed to the increased hydrophilicity of the scaffolds after addition of PGS. Thus, As-spun PGS scaffolds did not only improve mechanical properties but also showed better cell attachment and proliferation. Similarly, primary cardiomyocytes seeded on the scaffolds showed good cell viability even after 3 weeks in culture. PGS:PCL scaffolds also provided good guidance to the seeded cardiomyocytes allowing them to spread on the fibers (data not shown).

IV. CONCLUSION

We have fabricated microfibrinous scaffolds from difficult to electrospin PGS pre-polymer without any thermal or UV crosslinking. Scaffolds showed improved mechanical properties with higher PGS concentration. We also demonstrated improved cell attachment and proliferation on PGS scaffolds as compared to pure PCL scaffolds without compromising the mechanical properties.

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REFERENCES

1. Donald OF, Leo QW, Gordana V-N. Geometry and force control of cell function. *Journal of Cellular Biochemistry*. 2009; 108(5):1047–1058. [PubMed: 19795385]
2. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat Biotech*. 2005; 23(1):47–55.
3. Wang YD, Ameer GA, Sheppard BJ, Langer R. A tough biodegradable elastomer. *Nature Biotechnology*. 2002; 20(6):602–606.
4. Webb AR, Yang J, Ameer GA. Biodegradable polyester elastomers in tissue engineering. *Expert Opinion on Biological Therapy*. 2004; 4(6):801–812. [PubMed: 15174963]
5. Yang J, Webb AR, Ameer GA. Novel citric acid-based biodegradable elastomers for tissue engineering. *Advanced Materials*. 2004; 16(6):511–516.
6. Fidkowski C, Kaazempur-Mofrad MR, Borenstein J, Vacanti JP, Langer R, Wang YD. Endothelialized microvasculature based on a biodegradable elastomer. *Tissue Engineering*. 2005; 11(1–2):302–309. [PubMed: 15738683]
7. Chen QZ, Bismarck A, Hansen U, Junaid S, Tran MQ, Harding SE, Ali NN, Boccaccini AR. Characterisation of a soft elastomer poly(glycerol sebacate) designed to match the mechanical properties of myocardial tissue. *Biomaterials*. 2008; 29(1):47–57. [PubMed: 17915309]
8. Sundback CA, Shyu JY, Wang YD, Faquin WC, Langer RS, Vacanti JP, Hadlock TA. Biocompatibility analysis of poly(glycerol sebacate) as a nerve guide material. *Biomaterials*. 2005; 26(27):5454–5464. [PubMed: 15860202]
9. Redenti S, Neeley WL, Rompani S, Saigal S, Yang J, Klassen H, Langer R, Young MJ. Engineering retinal progenitor cell and scrollable poly(glycerol-sebacate) composites for expansion and subretinal transplantation. *Biomaterials*. 2009; 30(20):3405–3414. [PubMed: 19361860]
10. Engelmayr GC, Cheng MY, Bettinger CJ, Borenstein JT, Langer R, Freed LE. Accordion-like honeycombs for tissue engineering of cardiac anisotropy. *Nature Materials*. 2008; 7(12):1003–1010.
11. Radisic M, Park H, Gerecht S, Cannizzaro C, Langer R, Vunjak-Novakovic G. Biomimetic approach to cardiac tissue engineering. *Philosophical Transactions of the Royal Society B-Biological Sciences*. 2007; 362(1484):1357–1368.
12. Radisic M, Park H, Martens TP, Salazar-Lazaro JE, Geng WL, Wang YD, Langer R, Freed LE, Vunjak-Novakovic G. Pretreatment of synthetic elastomeric scaffolds by cardiac fibroblasts improves engineered heart tissue. *Journal of Biomedical Materials Research Part A*. 2008; 86A(3):713–724. [PubMed: 18041719]
13. Gao J, Crapo P, Nerern R, Wang YD. Co-expression of elastin and collagen leads to highly compliant engineered blood vessels. *Journal of Biomedical Materials Research Part A*. 2008; 85A(4):1120–1128. [PubMed: 18412137]
14. Hacking SA, Khademhosseini A. Applications of Microscale Technologies for Regenerative Dentistry. *Journal of Dental Research*. 2009; 88(5):409–421. [PubMed: 19493883]
15. Motlagh D, Yang J, Lui KY, Webb AR, Ameer GA. Hemocompatibility evaluation of poly(glycerol-sebacate) in vitro for vascular tissue engineering. *Biomaterials*. 2006; 27(24):4315–4324. [PubMed: 16675010]
16. Wang YD, Kim YM, Langer R. In vivo degradation characteristics of poly(glycerol sebacate). *Journal of Biomedical Materials Research Part A*. 2003; 66A(1):192–197. [PubMed: 12833446]
17. Kumbhar SG, James R, Nukavarapu SP, Laurencin CT. Electrospun nanofiber scaffolds: engineering soft tissues. *Biomedical Materials*. 2008; 3(3):1–15.
18. Liao S, Li B, Ma Z, Wei H, Chan C, Ramakrishna S. Biomimetic electrospun nanofibers for tissue regeneration. *Biomed Mater*. 2006; 1(3):R45–R53. [PubMed: 18458387]
19. Mauck RL, Baker BM, Nerurkar NL, Burdick JA, Li WJ, Tuan RS, Elliott DM. Engineering on the Straight and Narrow: The Mechanics of Nanofibrous Assemblies for Fiber-Reinforced Tissue Regeneration. *Tissue Eng Part B Rev*. 2009; 15(2):171–193. [PubMed: 19207040]
20. Soffer L, Wang XY, Mang XH, Kluge J, Dorfmann L, Kaplan DL, Leisk G. Silk-based electrospun tubular scaffolds for tissue-engineered vascular grafts. *Journal of Biomaterials Science-Polymer Edition*. 2008; 19(5):653–664. [PubMed: 18419943]

21. Li CM, Vepari C, Jin HJ, Kim HJ, Kaplan DL. Electrospun silk-BMP-2 scaffolds for bone tissue engineering. *Biomaterials*. 2006; 27(16):3115–3124. [PubMed: 16458961]
22. Ma Z, Kotaki M, Inai R, Ramakrishna S. Potential of nanofiber matrix as tissue-engineering scaffolds. *Tissue Eng*. 2005; 11(1–2):101–109. [PubMed: 15738665]
23. Hwang CM, Khademhosseini A, Park Y, Sun K, Lee SH. Microfluidic chip-based fabrication of PLGA microfiber scaffolds for tissue engineering. *Langmuir*. 2008; 24(13):6845–6851. [PubMed: 18512874]
24. Hwang CM, Park Y, Park JY, Lee K, Sun K, Khademhosseini A, Lee SH. Controlled cellular orientation on PLGA microfibers with defined diameters. *Biomedical Microdevices*. 2009; 11(4): 739–746. [PubMed: 19242806]
25. Ifkovits JL, Padera RF, Burdick JA. Biodegradable and radically polymerized elastomers with enhanced processing capabilities. *Biomedical Materials*. 2008; 3(3):1–8.
26. Nijst CLE, Bruggeman JP, Karp JM, Ferreira L, Zumbuehl A, Bettinger CJ, Langer R. Synthesis and characterization of photocurable elastomers from poly(glycerol-co-sebacate). *Biomacromolecules*. 2007; 8(10):3067–3073. [PubMed: 17725319]
27. Yi F, Lavan DA. Poly(glycerol sebacate) nanofiber scaffolds by core/shell electrospinning. *Macromolecular Bioscience*. 2008; 8(9):803–806. [PubMed: 18504802]
28. Ifkovits JL, Devlin JJ, Eng G, Martens TP, Vunjak-Novakovic G, Burdick JA. Biodegradable Fibrous Scaffolds with Tunable Properties Formed from Photo-Cross-Linkable Poly(glycerol sebacate). *Acs Applied Materials & Interfaces*. 2009; 1(9):1878–1886. [PubMed: 20160937]
29. Balguid A, Rubbens MP, Mol A, Bank RA, Bogers A, Van Kats JP, De Mol B, Baaijens FPT, Bouten CVC. The role of collagen cross-links in biomechanical behavior of human aortic heart valve leaflets - Relevance for tissue engineering. *Tissue Engineering*. 2007; 13(7):1501–1511. [PubMed: 17518750]

Fig. 1.
Scanning electron microscopic images of fibrous scaffolds. Scale bar=10 μm .

Fig. 2.

A. Scanning electron microscopic images of cells seeded on the fibrous scaffolds. Scale bar=10 μm. Cells remain rounded on PCL scaffolds whereas they showed spread morphology on PGS:PCL scaffolds. B. Cell proliferation on fibrous scaffolds.

Table 1

Effect of PGS:PCL ratio on the fiber diameter

PGS:PCL ratio	Fiber Diameter (μm)
0:1	2.8 ± 0.03
2:1	4.0 ± 0.11
3:1	6.1 ± 0.17
5:1	6.77 ± 0.27

Table 2

Mechanical properties of scaffolds

PGS:PCL ratio	EM (MPa)	UTS (MPa)	UE (%)
0:1	11.6 ± 2.6	1.6 ± 0.37	626 ± 15
2:1	8.5 ± 1.6	1.8 ± 0.02	443 ± 66
3:1	8.6 ± 1.3	1.41 ± 0.10	363 ± 60
5:1	34.4 ± 1.9	2.45 ± 0.16	566 ± 55