ELECTROSPUN BLENDS OF NATURAL AND SYNTHETIC POLYMERS AS SCAFFOLDS FOR TISSUE ENGINEERING

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ABSTRACT

Engineering functional three-dimensional (3-D) tissue constructs for the replacement and/or repair of damaged native tissues using cells and scaffolds is one of the ultimate goals of tissue engineering. In this study, non-woven fibrous scaffolds were electrospun from the synthetic biodegradable polymer poly(lactic-co-glycolic acid) (PLGA) and natural proteins, gelatin (denatured collagen) and elastin. In the absence of cross-linking agent, the average PGE fiber diameter increased from 347 ± 103 nm to 999 ± 123 nm upon wetting as measured by scanning electron microscopy. Rat bone marrow stromal cells (rBMSC) were used paradigmatically to study the 3-D cell culture properties of PGE scaffolds. Consistent with the observed properties of the individual fibers, PGE scaffolds initially swelled in aqueous culture medium, however rBMSC seeded PGE scaffolds contracted to < 50% of original size. Time course histological analysis demonstrated uniform seeding of rBMSC into PGE scaffolds and complete cell penetration into the fibrous architecture over 4 weeks of *in vitro* culture.

Key words: Electrospinning; Nanofibrous scaffold; PLGA, Gelatin; Elastin, Tissue Engineering

INTRODUCTION

Extracellular matrix proteins are important modulators of the cellular microenvironment, making them attractive choices for the fabrication of tissue-engineered scaffolds [1-3]. Electrospinning is an attractive process for fabricating scaffolds for tissue engineering applications due to the simplicity of the process and the ability to generate microscale and nanoscale features with synthetic and natural polymers [4]. One limitation of electrospun scaffolds fabricated with single materials is the high fiber density and the resultant fishnet effect, with the fiber density in electrospun mats is often too high to allow for ingrowth of cells. A major drawback of fibrous protein scaffolds is the need for crosslinking in order to prevent rapid hydrolysis of the delicate fibers. Possible solutions include the use of composite scaffolds containing heterogeneous fibers of natural and synthetic origin which display differential gel-solid state transition upon introduction to aqueous medium in order to create fiber-hydrogel composites. With that, the solid state synthetic polymer material can function as a fibrous backbone while non-crosslinked protein hydrogel components act as promoters of cellular attachment and growth. In this study, we dissolved PLGA, gelatin and elastin in the same HFP solvent and electrospun this mix into a 3-D fibrous mat. Upon exposure to aqueous medium the resultant composite PGE scaffold became a stable fiber-laden hydrogel with suitable mechanical properties without need for chemical cross-linking. Furthermore, PGE scaffolds displayed superior capacity to support 3-D tissue-like assembly when compared to fiber scaffolds constructed from the individual components. Thus, this novel composite scaffold exhibited some of the advantages of the individual components, providing proof of concept for the engineering design principles.

METHODOLOGY

A. Electrospinning

All polymer materials, Poly(lactide-co-glycolide) (PLGA), gelatin and alpha-elastin (in abbreviation as PGE), were dissolved in

1,1,1,3,3,3 Hexafluoro-2-Propanol (HFP) at concentrations of 30%, 8% and 20% (w/v), respectively, and then mixed as a blend PGE solution at the ratio of 2:3:5 (v/v/v). Fibers were electrospun at voltage of 10 kV, air gap distance of 15 cm, and delivery rate of 1.5 ml/h.

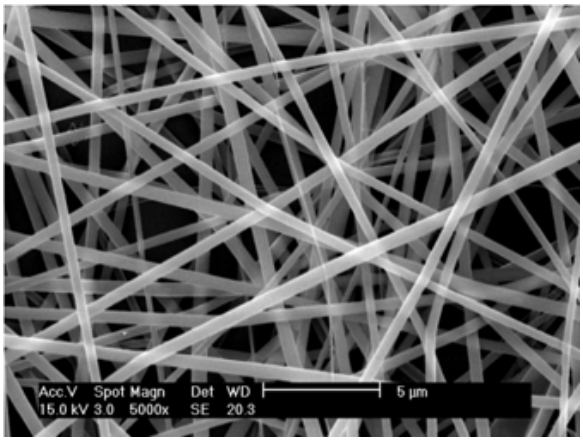
B. Cell Culture

PGE scaffolds were placed on an orbital shaker and seeded overnight using a 500,000/mL suspension of rat bone marrow stromal cells (rBMSC) in complete cell culture medium. rBMSCs were maintained essentially in a 50/50 mixture of alpha-MEM and Ham's F-12 media containing 10% FBS and antiobiotics was used. The BMSC-seeded PGE constructs were cultured under static conditions at 37 ºC in a 5% CO_2 for up to 28 days, with samples taken at 24 hours, 7 days, and 28 days to evaluate cell ingrowth over an extended *in vitro* culture period.

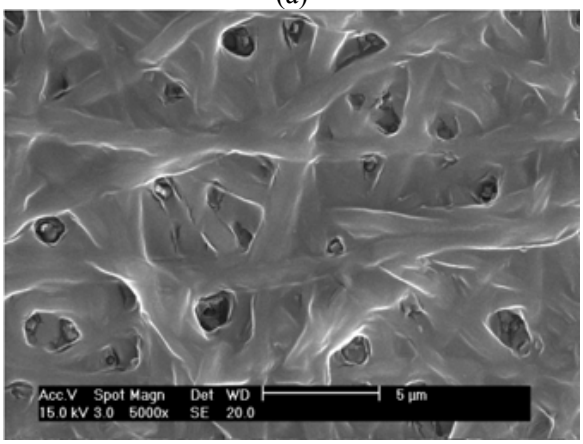
RESULTS AND DISCUSSION

Based on our previous studies on electrospinning parameters [5], PGE was electrospun into mats approximately 0.5 mm thick at 10 kV and 1.5 ml/h flow rate with 15 cm air gap distance. Resultant PGE fibers were homogenous in appearance (Fig 1a), suggesting that the PGE fibers are hybrid fibers composed of PLGA, gelatin, and elastin, rather than a mixture of homogeneous PLGA, gelatin, and elastin fibers. This observation is in stark contrast to previous reports Boland et al. who report heterogeneous/phase separated deposition of co-spun elastin and collagen fibers. In our experiments, the average fiber diameter of PGE was 347 ± 103 nm (n=30). The diameter of homogeneous PLGA fibers was 372 ± 112 nm, gelatin was 349 ± 97 nm, and elastin was 605 ± 102 nm at the same electrospinning condition, which indicated that the fiber sizes did not change significantly with co-electrospinning.

To test the behavior of electrospun PGE scaffolds in an aqueous environment, the fibrous mats were soaked for 1 h in distilled water. PGE scaffolds swelled, resembling an opaque hydrogel and becoming more three dimensional than the fibrous mats obtained following electrospinning.



(a)



(b)

Fig. 1. SEM graphs of PGE scaffold (a) before and (b) after soaked in dd H_2O.

SEM images, taken after drying the soaked PGE mats clearly indicated swelling of the fibers Fig. 1(b). The average diameter of soaked PGE fibers is 999 ± 123 nm (n=10), which is much larger than the native PGE fibers ($p<0.01$) (Figure 2). Swelling of electrospun fibers and formation of fiber-hydrogel composites, specifically of tertiary blends of synthetic biodegradable polymers and natural extracellular matrix proteins has never been reported. As an explanation for this behavior we propose that the blend PGE is arranged in such a way that PLGA serves as a backbone while the water-soluble ECM molecules gelatin/elastin are arranged, facing the aqueous phase adsorbed on it.

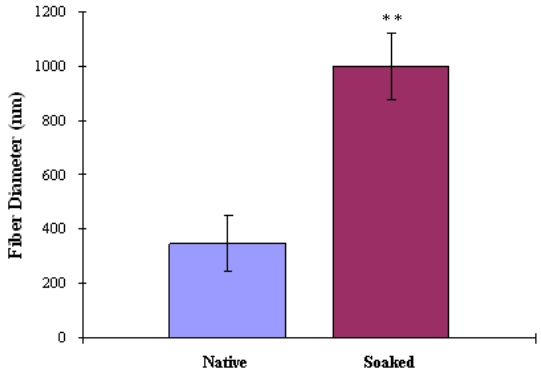


Fig. 2. Fiber sizes of PGE scaffold before and after soaked in water.

After overnight seeding of rBMSC into PGE scaffolds on an orbital shaker, PGE constructs were transferred to fresh cell culture plate and cultured for a period of 4 weeks. Upon seeding with rBMSC, the PGE scaffolds contracted to < 50% of the original swollen size. Histological characterization of the PGE constructs indicated rBMSCs uniformly and efficiently penetrated into the scaffolds following the approximately 24 hour seeding period (Fig 3).

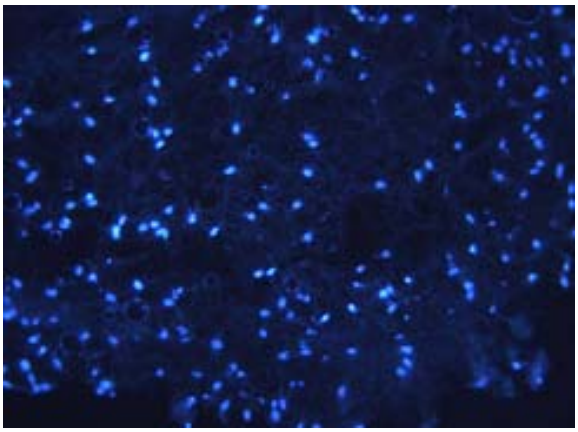
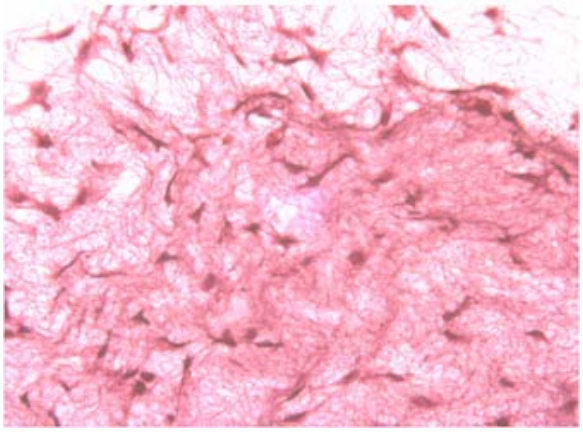
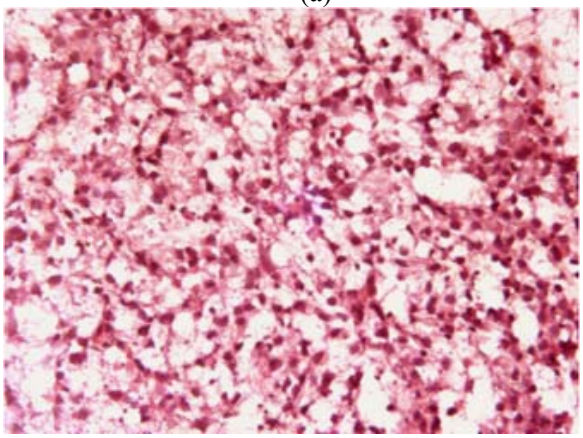


Fig. 3. Bisbenzimide staining of 10 μm sections of PGE scaffolds prepared following the 24 hour seeding period (400x).

Fig. 4 shows histological images of the constructs at 7 days and 4 weeks post-seeding. rBMSCs penetrated into the fibrous scaffold after only 24 hours seeding and continued to proliferate over time as evidenced by increasing cell densities in histological sections.



(a)



(b)

Fig. 4. Histological images of rBMSC in PGE fibrous scaffolds at
(a) 7 days; (b) 28 days post-seeding.

In summary, we have provided data characterizing the structural and mechanical properties and 3-D cell culture applicability of a novel composite fibrous scaffold composed of PLGA, gelatin, and elastin. PLGA is a gold standard biomaterial that has seen wide applications in tissue engineering and drug delivery confirming superior biocompatibility as compared with most synthetic polymers. For this reason, PLGA was selected as the synthetic component of our engineered matrix. In addition, we hypothesize that the synthetic polymer components of such composite matrices, in this case PLGA, could be used as a delivery vehicle for proteins or DNA as needed to facilitate the molecular events needed for generation of given tissue. Structural proteins such as gelatin and elastin fibrils are an important factor for the mechanical properties of the ECM as well as for

or cell attachment and migration. In an attempt to mimic these properties, our fibrous scaffolds are engineered to be porous, pliable, and elastic, so that cells will be able to integrate and/or push the ECM-like fibers aside as they grow into the scaffolds. This design aim was realized, as evidenced by the formation of a fiber-hydrogel composite with mechanical properties similar to homogeneous elastin that allowed for cell penetration while retaining gross physical properties that would be required for eventual transplantation of constructs generated *in vitro*.

The swelling and hydrogel formation within the PGE matrix upon exposure to aqueous medium is in our opinion what facilitates the superior tissue-like assembly observed within these matrices After 28 days of *in vitro* culture the interior of the scaffold contains large cell numbers. These images demonstrate complete penetration of cells with high density throughout the cross-section of an electrospun fiber scaffold. Remarkably, there is no evidence of a necrotic core as is often seen in attempts to engineer macroscopic tissue constructs *in vitro*, indicating that the porosity of the scaffold might facilitate transport of nutrient into the scaffold and removal of toxic waste. We surmise that the robust cellular penetration observed in the absence of overt necrosis is due to the malleability of the PGE matrix. This scaffold design is a prototype for future composites that will combine the benefits of engineered synthetic materials and natural extracellular matrix proteins.

CONCLUSION

Electrospinning provides an efficient approach to fabricating biomimetic scaffolds from both natural and synthetic polymer and proteins for tissue engineering. In this paper, we electrospun a fibrous composite PGE scaffold composed of PLGA, gelatin, and elastin. The advantage of this scaffold was the beneficial characteristics of each individual component synergizing to form a composite with more superior three dimensional cell culture properties. This conclusion is corroborated by histological analyses indicating that rBMSCs attach, migrate, and proliferate within the PGE matrix, resulting in extremely high cell densities by 4 weeks of *in vitro* culture. These scaffolds would have wide applicability in a number of tissue engineering systems due to their ability to support dense cell growth, thus having the ability to deliver extremely high cell numbers. This novel approach to generating composite scaffolds of natural and synthetic biomaterials affords tissue engineers the ability to meet all necessary design criteria in fabricating scaffolds for a given application.

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REFERENCES

[1] Lu Q, Ganesan K, Simionescu DT, Vyavahare NR. Novel porous aortic elastin and collagen scaffolds for tissue engineering. Biomaterials. 2004, 25(22):5227-5237.

[2] Buijtenhuijs P, Buttafoco L, Poot AA, Daamen WF, van Kuppevelt TH, Dijkstra PJ, de Vos RA, Sterk LM, Geelkerken BR, Feijen J, Vermes I. Tissue engineering of blood vessels: characterization of smooth-muscle cells for culturing on collagen-and-elastin-based scaffolds. Biotechnol Appl Biochem. 2004, 39(Pt 2):141-149.

[3] Kim BS, Mooney DJ. Scaffolds for engineering smooth muscle under cyclic mechanical strain conditions. J Biomech Eng. 2000, 122(3):210-5.

[4] Nair LS, Bhattacharyya S, Laurencin CT. Development of novel tissue engineering scaffolds via electrospinning. Expert Opin Biol Ther. 2004, 4:659-68.

[5] Li M, Mondrinos MJ, Gandhi MR, Ko FK, Weiss AS, and Lelkes PI, Electrospun Protein Fibers as Matrices for Tissue Engineering, Biomaterials, in press, 2005.