BIO-INSPIRED, NANO-STRUCTURED POLYMERS FOR USE IN SOFT TISSUE REPLACEMENT APPLICATIONS

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INTRODUCTION

Previous work in the area of tissue-constructs for soft tissue replacements has involved the use of matrices derived from natural extra-cellular proteins, such as collagen; these scaffolds are often limited by their poor mechanical stability, can provoke adverse tissue and immune responses, and may elicit undesired cell behavior [1]. The use of synthetic biodegradable polymers in such constructs has been the major focus of recent work due to ease of manufacturing, reproducibility, and designability of these polymers. For example, Atala, et al. [1] have reported that the construction of urothelial and bladder smooth muscle structures *in vivo* might be successfully achieved by the use of biodegradable polymers like poly(glycolic acid), which serves to deliver the appropriate cells at the implant site and is replaced within a certain period of time once the body fully resorbs the material. The present study has focused on the development and enhancement of the properties of such synthetic implants in an effort to minimize complications that exist in current polymer formulations. In order to complete this task, and since all proteins found in the body have nanometer scale dimensions, we fabricated novel, nano-structured, biodegradable materials for use as substrates for soft tissue replacement constructs and tested (*in vitro*) the cytocompatibility properties of these biomaterials. We used an approach based on biodegradable polymers like poly-lactic-co-glycolic acid and have aimed to design biomaterial constructs capable of mimicking the *in vivo* properties of several soft tissues and organs.

MATERIALS AND METHODS

Novel Bio-inspired Material Fabrication

Bio-inspired copolymers of Poly (lactic acid) and Poly (glycolic acid) (PLGA; 50:50 wt%; Polysciences, Inc.) were synthesized by heat-dissolving the co-polymer (0.5 g) in chloroform. The heat-dissolving process lasted for approximately 40 minutes at temperatures around (but below) 60 °C. The dissolved copolymers were left partially covered at room temperature overnight and were vacuum dried (at 15 inches gauge pressure) for 48 hours to allow the chloroform to evaporate. Polymer scaffolds (1 cm X 0.5 cm X 0 .05 cm) were cut from the resulting bulk polymer film.

Fiber Dimension Reduction

In order to reduce polymer fiber dimensions into the nanometer regime, PLGA polymer scaffolds were soaked for various amounts of time in select concentrations of NaOH. After chemical treatment, all substrates were rinsed in de-ionized water until reaching a stable pH of 7.0. The substrates were then sterilized by UV light for 1 hour on each side; finally, polymers were soaked overnight in 70% ethanol. Scanning electron micrograph pictures were obtained for each substrate in order to visualize the resulting fiber dimensions.

Cell Culture

Ovine bladder smooth muscle cells (OBSMC) were isolated from neonatal bladder muscularis using techniques that we have previously described [2] and were characterized by the expression of α-smooth muscle actin. These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 5% penicillin/streptomycin under standard cell culture conditions (that is, a sterile, 37° C, humidified, 5% CO_2/95% air environment). Rat aortic endothelial cells (RAEC) and rat aortic smooth muscle cells (RASMC) were purchased form VEC Technologies and were cultured in either MCDB-131 Complete Medium or Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S), respectively.

Cell Adhesion and Proliferation on PLGA

Polymer scaffolds were prepared as previously described to obtain conventional (control), sub-micron structured, large nano-structured, and small nano-structured diameter fibers. Etched glass cover slips were used as reference substrates. Cells were seeded (3,500 cells/cm^2) onto each substrate and incubated under standard cell culture conditions in DMEM for either 4 hours (for adhesion experiments) or 1 day, 3 days and 5 days (for proliferation

experiments). All experiments were run in triplicate and were repeated a minimum of three separate times.

At the end of the prescribed time period, cells were fixed with 4% formaldehyde and stained with Hoechst 33258 (Sigma). The number of cells in each of five random fields per substrate was counted using a fluorescence microscope, averaged, and recorded as cells/cm^2. Numerical data were analyzed statistically using student t-tests; data was considered significant at $p < 0.05$.

RESULTS

PLGA Fiber Dimension Reduction

Scanning Electron Micrographs of the substrates indicated that fiber dimensions were reduced as NaOH concentration and treatment times were increased. Moreover, images of the control (NaOH untreated) and nano-structured (NaOH treated) PLGA samples provided the first evidence that treating scaffolds with 10 N NaOH for 1 hr resulted in reduction of PLGA fiber diameters into nanometer regime.

Cell Adhesion and Proliferation on PLGA

Results from cell adhesion experiments indicated that nano-fibered PLGA enhanced cell adhesion. For example, compared to conventional micron-fibered controls, bladder smooth muscle cell adhesion was statistically greater ($p < 0.01$) on polymer formulations with nanometer fiber sizes compared to that on conventional PLGA films. In contrast, no significant differences were observed between the number of cells adherent to large nano-structured PLGA (d≅100nm-1μm) and small nano-structured PLGA (d≅50nm-100nm) after 4 hours.

Similarly, results from proliferation experiments indicated that cell proliferation was greatly enhanced ($p <0.01$) on both large and small nano-fibered PLGA substrates compared to both conventional (micron-dimensional) PLGA substrates and reference substrates (etched glass) at each time period. Proliferation of all cells on nano-structured substrates was also significantly increased ($p <0.01$) after 3 and 5 days of incubation compared to proliferation after 1 day of incubation, providing evidence that the nano-structured polymers were capable of sustaining cell growth at all time points tested in the present study.

DISCUSSION

Gao et al [3] previously described the hydrolysis procedure by which fibers in poly(glycolic) acid (PGA) meshes are reduced. Briefly, hydroxide anion from NaOH hydrolyzes the ester bond on the surface of the PGA mesh, thereby exposing carboxylic acid and hydroxyl groups by breaking the polymer chain. This process happens at various points, leading to multiple hydrolysis depending upon access of hydroxide ions to the polymer chains. Such hydrolysis may result in break down of the polymer into oligomeric or monomeric forms, whereby NaOH is able to dissolve portions of the polymer fibers.

Since PLGA is also a poly(ester), we expected similar interactions between hydroxide ions of NaOH and the ester bonds of PLGA. We therefore treated PLGA films with various NaOH solutions and have succeeded in achieving, for the first time, nano-dimensionality at the substrate-surface (confirmed by SEM pictures)

Furthermore, we tested the effects of fiber-dimension reductions to cellular responses on PLGA substrates. The results from our *in vitro* work to date have provided the first evidence that cellular responses (such as adhesion and proliferation) are enhanced as polymer fiber dimensions are reduced to the nanometer range. This information clearly indicates that nano-fibered PLGA is cytocompatible; more importantly, nano-dimensional substrates are more cytocompatible than conventional substrates.

CONCLUSION

Our study has, for the first time, developed PLGA polymer substrates with nano-fiber dimensionality for use in soft tissue replacement therapies. In addition, we have shown that cellular responses (such as adhesion and proliferation) are enhanced in nano-fibered polymeric substrates compared to conventional micron-fibered substrates. Improved adhesion and proliferation on the nano-dimensional substrates suggest that, once implanted, the tissue constructs will be highly biocompatible to the native tissue. In summary, we report that nano-dimensional polymers show great potential as novel implant materials that can be used for successful tissue engineered soft tissue replacement constructs.

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REFERENCES

1. Atala A, Freeman MR, Vacanti JP, Shepard J, and Retik AB, 1993, "Implantation In Vivo and Retrieval of Artificial Structures Consisting of Rabbit and Human Urothelium and Human Bladder Muscle," *The Journal of Urology*, Vol. 150, pp. 608-612.

2 Haberstroh KM, Kaefer M, Retik AB, Freeman MR, and Bizios R., 1999, "The Effects of Sustained Hydrostatic Pressure on Select Bladder Smooth Muscle Cell Functions," *The Journal of Urol*ogy, Vol. 162(6**)**: pp. 2114-2118.

3. Gao J, Niklason L, and Langer R., 1998, "Surface Hydrolysis of Poly(glycolic acid) Meshes Increases the Seeding Density of Vascular Smooth Muscle Cells," *The Journal of Biomedical Materials Research,* Vol. 42(3), pp. 417-424.