Multilayered Nanofibrous Scaffolds With Spatiotemporally-Controlled Molecule Signals
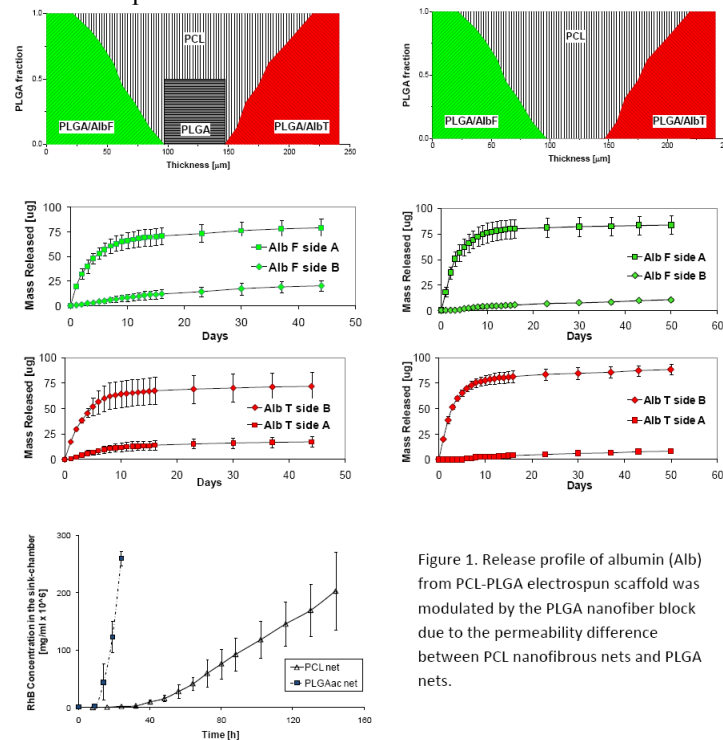
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Statement of Purpose: Tissue regeneration requires multifunctional scaffolds that not only serve temporary mechanical function and physical support for cell growth, but also stimulate sequential regenerative events involving multiple tissue-specific cell types. In particular, the use of stem cells for tissue regeneration, often aiming to simulate stepwise developmental events, involves precise spatiotemporally-controlled actions of multiple soluble factors to induce cell differentiation, maturation and matrix production. Electrospun polymer scaffolds have become one major direction in tissue engineering because these materials provide biodegradable matrices with high surface area for cell adhesion, high porosity for nutrient transfer, and opportunity of controlled molecule delivery. We thus aim to develop functional material model systems as well as theoretical models for spatially-controlled multipulse biomolecular delivery to effectively recapitulate molecular environment during development for stem cell differentiation and tissue formation.

Methods: The electrospun composite with the compositional gradient of distinct materials across the composite thickness was prepared through programming the sequential and/or simultaneous electrospinning processes of poly(ε-caprolactone) or PCL and 50:50 poly(D,L-lactide-co-glycolide) or PLGA on a rotating mandrel collector, using a modified double-electrospinning system previously described by us.[1] Briefly, layers of PCL nanofibers, intermixed PCL/PLGA nanofibers, and PLGA fibers were deposited in sequence on the grounded cylindrical aluminum collector. The design of the compositional patterns along the scaffold thickness was achieved by controlling 3D movements of a collector, in coordination with separately programmed spinning processes. The cylindrical collector rotated at a constant velocity by a brushless rotating electric motor. Each spinneret placed at the opposite side of the rotating collector was perpendicularly oriented with respect to the principal axis of the collector. The feed rate of each solution varied independently via separate syringe pumps during the course of deposition. The electrospinning flow rates were determined before for each solution in order to form defect-free nanofibers. These flow rates, 0.6 mL/h for PLGA fibers and 1.1 mL/h for PCL fibers, defined the maximum flow rates. The collector geometry, the concentration of polymer solutions, and apparent density of pure PCL and PLGA electrospun nets were used to establish a correlation between the feed rate and the deposition rate of each polymer per unit of surface area. The final nets were characterized with confocal microscopy, scanning electron microscopy and differential scanning calorimeter. Nanofibers were also used to impregnate proteins, e.g. fluorescently-labeled albumin for characterizations and quantitative analysis or modeling, as well as VEGF and TGF-b for cell differentiation and proliferation studies.

Results: Material characterizations with albumin have demonstrated micropatterns of polymeric nanofibers can program the spatial and temporal release profiles of the protein, which is based on the material degradation rate and hydrophobicity, responsible for the long-term and short-term release profiles, respectively. PLGA and PCL are characterized with different hydrophobicity, essential in controlled-releasing scaffold design (Figure 1). Albumin-growth factors are further incorporated into micropatterned nanofibrous scaffold to stimulate biochemical signaling for cell differentiation, proliferation and matrix production.

Figure 1. Release profile of albumin (Alb) from PCL-PLGA electrospun scaffold was modulated by the PLGA nanofiber block due to the permeability difference between PCL nanofibrous nets and PLGA nets.

Conclusions: Micropatterning electrospun nanofibers with biomaterials of varying hydrophobicity and/or degradation, provides not only control over biomolecule release rates at various stages but also modulation on molecule diffusion and porosity throughout the scaffolds, thus providing a robust technique for spatiotemporal control over cell activity and tissue regeneration . Effective interplay between scaffolding materials and controlled release strategies is therefore critical.

(1) Bonani, W.; Maniglio, D.; Motta, A.; Tan, W.; Migliaresi, C. J. Biomed. Mater. Res., Part B 2011, 96B, 276−286