Nanofiber Gradient Pattern For Spatiotemporal Controlled Release of Biomolecules From Vascular Graft

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Statement of Purpose: Autologous vessels are the gold standard for small-diameter (<6 mm) vascular bypass; however, many patients lack suitable autologous tissues due to diseases or prior vein harvest. As an alternative, synthetic vascular grafts made from bioinert synthetic materials are currently in use. The high long-term failure rate of these materials in the replacement of small vessels is known to be associated with the lack of physiological signals to vascular cells causing adverse hemodynamic, inflammatory or coagulatory events. Aimed at constructing a bioresorbable scaffold to guide vascular regeneration *in vivo*, we need scaffolds that provide not only proper mechanical support, but also precise spatiotemporal dynamics of inductive cues for collaborative efforts of various cells. Because vascular regeneration is characterized by sequential cell events involving a number of molecules that target at different vascular cell types and regulate varied cell activities, biomolecules must be carefully regulated spatially and temporally for controlled cell-graft interactions. To that end, we have developed double-electrospinning to obtain interpenetrating networks of nanofibers made from polymers with different degradation rates. The nanofibers are patterned in a tailored proportion forming heterogeneous gradients of materials and functional biomolecules. The nanofiber construct together with porous collagen-based gel is then used to prepare a multilayer vascular graft.

Methods: Poly ε-caprolactone (PCL) and poly lactic-co-glycolic acid (PLGA) are co-electrospun with a custom-made double-electrospinning apparatus into nanofibers that are structurally similar to ECM protein fibers. Fluorescent dyes or bioactive molecules are encapsulated in the nanofibers. Using a spatial release chamber, time-lapse degradation tests and molecule release assays are performed with an isotonic phosphate solution on the biomolecule-impregnated nanocomposite constructs. Vascular cells are used to evaluate the bioactivity of the biomolecules.

Results: Confocal imaging of the nanofiber scaffold demonstrate that the composite made from dye-impregnated PCL and PLGA are characterized by interpenetrating nanofiber structure with heterogeneous nanofiber and molecule composition. Its compositional profile and concentration gradients along the scaffold thickness is closely correlated with the design pattern (Fig 1A). Characterization results show that the release of dye molecules from these nanocomposites can be controlled spatially, temporally and sequentially (Fig 1B and 1C). The hybrid nanocomposites also demonstrate the capability of precise spatio-temporal control over releases of bioactive molecules including heparin and biomolecules through different degradation kinetics of polymer nanofibers. Furthermore, the migration and differentiation of vascular endothelial cells in response to the biomolecule release profiles are studied.

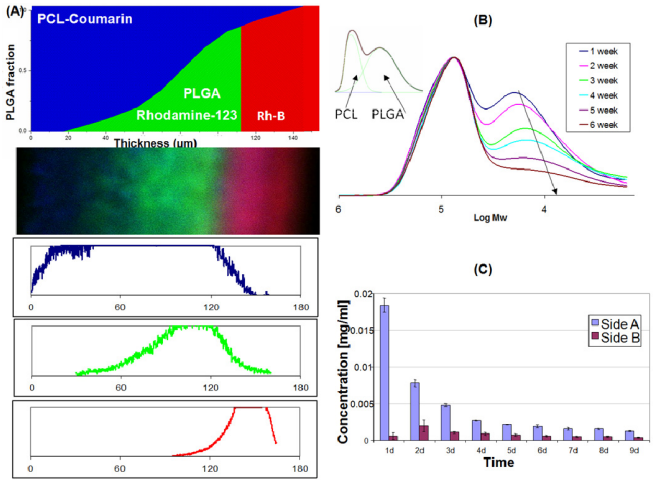

Fig 1. (A) Compositional gradient profile over the thickness of the material is demonstrated with fluorescent molecules-encapsulated nanofibers. (B) Degradation profiles of PCL-PLGA (50/50) interpenetrating nanofiber composite are characterized with GPC. (C) Spatial control over the molecule release

Conclusion: The newly-developed hybrid nanofiber composites are characterized by heterogeneous polymer composition and molecule-nanofiber gradients. The nanofiber pattern provides the construct with the capability of precise spatio-temporal control over molecular release and biodegradable kinetics. Such precise control is biologically essential for proper activities and functions of vascular cells, and the ultimate success of vascular engineering. The nanofiber construct, together with the multilayer design of vascular graft, forms a novel platform that offers a biomimetic molecule environment for vascular regeneration.