Interaction of Mesenchymal Stem Cells with Electrospun Composite Scaffolds to Assess Potential for Bone Regeneration

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Statement of Purpose: The use of autografting in surgeries requiring bone grafts has multiple drawbacks, including limited available tissue and secondary surgery sites. In order to replace autografts, many have turned toward the process of electrospinning due to its ability to fabricate a nanofibrous mesh that is highly representative of native extracellular matrices[1]. Natural bone matrix is mainly comprised of collagen I fibers mineralized with the calcium phosphate hydroxyapatite (HA). Our laboratory is developing a synthetic bone graft material by electrospinning scaffolds composed of these two native bone molecules, in combination with a polymer, polycaprolactone (PCL), which provides tensile strength. Previously we determined that these scaffolds have an average fiber diameter of 180 ± 50 nm (comparable to that of natural bone collagen fibers) and uniform dispersion of HA particles throughout fiber length. In this study, we evaluated human mesenchymal stem cell (hMSC) responses to these bone-mimetic scaffolds (col/HA/PCL), as compared with scaffolds composed of 100% PCL (PCL). We also monitored bone formation on scaffolds implanted into rat tibiae.

Methods: Two scaffold formulations were produced by electrospinning as described previously[2]: 100% PCL and 50% PCL + 30% Collagen I + 20% nanoHA. Briefly, composite solutions were made by adding hexafluoroisopropanol to each mixture such that the solid weight was 7.5% of the total solution weight. The composite solutions were then loaded in a syringe and connected to a high voltage source, placed a set distance from a grounded collecting plate. Protein absorption was accomplished by soaking scaffolds in FBS, BMP-2 solution or inserting into rat tibial osteotomies for 30 minutes. Protein absorption was verified by western blotting for specific proteins. hMSC response was evaluated *in vitro* via scanning electron microscopy (SEM), fluorescent morphology, MTT assay or osteocalcin ELISA. Rat tibial osteotomies were used as *in vivo* models, and tissue sections were evaluated with Goldner's Trichrome to visualize bone mineral.

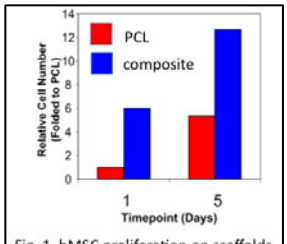

Fig. 1. hMSC proliferation on scaffolds was measured by MTT assay

Results: To test the hypothesis that incorporation of collagen I and HA in electrospun scaffolds has a beneficial effect on MSC behavior, initial cell adhesion, cell spreading and cell survival/proliferation were compared on composite vs PCL scaffolds. As measured by SEM and live cell imaging of hMSCs labeled with Green Fluorescent Protein, MSCs adhered, spread and proliferated better on the composite scaffolds (not shown). These results were also verified quantitatively using an MTT assay (Fig 1). We then examined the ability of electrospun scaffolds to adsorb pro-adhesive proteins from the bone tissue microenvironment, given that such proteins can enhance cell adhesion to biomaterials. We found that composite scaffolds adsorbed more of the pro-adhesive proteins fibronectin and vitronectin than PCL when implanted into rat tibial osteotomies or soaked in Fetal Bovine Serum (Fig 2). Next, we hypothesized that the higher adsorptive capacity of composite scaffolds could be exploited to deliver factors that promote bone formation in vivo by stimulating osteoblastic differentiation of MSCs. We observed that composite scaffolds showed an increased adsorption of recombinant human Bone Morphogenic Protein-2 (rhBMP-2), one of the most potent inducers of osteoblastic differentiation known. The bioactivity of the absorbed rhBMP-2 was confirmed by seeding hMSCs onto scaffolds coated with rhBMP-2, and measuring markers of osteoblastic differentiation, such as osteocalcin. As shown in Fig. 3, the presence of rhBMP-2 on electrospun scaffolds led to an increase in osteocalcin secretion by hMSCs on composite scaffolds in comparison to PCL. Finally, scaffolds were implanted in tibial osteotomies and it was found that composite scaffolds stimulated greater new bone formation in comparison to PCL (not shown).

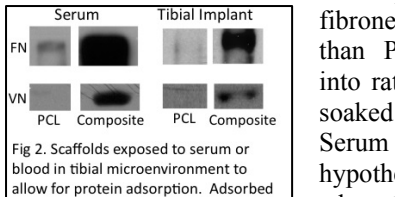

Fig 2. Scaffolds exposed to serum or blood in tibial microenvironment to allow for protein adsorption. Adsorbed proteins were solubilized and subjected to western blotting

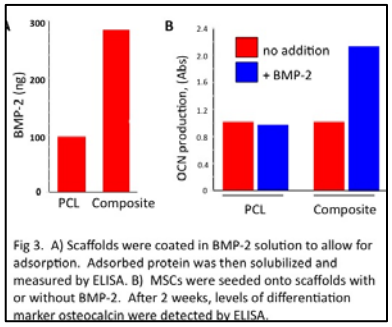

Fig 3. A) Scaffolds were coated in BMP-2 solution to allow for adsorption. Adsorbed protein was then solubilized and measured by ELISA. B) MSCs were seeded onto scaffolds with or without BMP-2. After 2 weeks, levels of differentiation marker osteocalcin were detected by ELISA.

Conclusions: By electrospinning a composition of the polymer PCL, collagen I and HA, we are developing a biomaterial that mimics the natural matrix of bone at the nanoscale. These scaffolds not only have favorable mechanical properties, but also contain biological cues that help MSCs grow and differentiate into osteoblasts. In addition, our studies suggest that the high adsorptive ability of these scaffolds may facilitate the delivery of specific signaling molecules that stimulate the bone healing process. We anticipate that these scaffolds may ultimately serve as a suitable counterpart or replacement for autografted bone tissue.

References:

1) Navarro M. et al. J. R. Soc. Interface. 2008;5:1137-58.
2) Catledge, SA. et al. Biomed Mater, 2007;2: 142-150