In Vitro and *In Vivo* Study to Evaluate Polycaprolactone/ Hydroxyapatite/ Collagen I Electrospun Scaffolds for Bone Tissue Engineering Applications

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Statement of Purpose: Tissue engineered grafts developed from a sample of bone marrow derived cells offer the promise of eliminating the need for autogenous bone harvesting. We have previously reported the development of biomimetic scaffolds that provide temporary structure to osteoblasts as a strategy for creating grafts *in vitro*.[1] The scaffolds are fibrous composites composed of collagen I, hydroxyapatite nanoparticles (nanoHA), and polycaprolactone (PCL), produced by electrospinning. The purpose of this study is to evaluate the biocompatibility and osseointegration of various formulations composed of these three biomaterials.

Methods: Nanofibrous scaffolds were produced by the technique of electrospinning. The following composite mixtures were made (percentage of total solid weight): PCL (80%) + nanoHA (20%), collagen (80%) + nanoHA (20%), and PCL (50%) + collagen (35%) + nanoHA (15%). The resulting samples were randomly arranged fibers deposited as a sheet with estimated maximum thickness between 50-100 μm on aluminum foil. Mechanical properties of scaffolds were evaluated by nanoindentation.

For *in vitro* studies, human mesenchymal stem cells (hMSCs) were seeded onto scaffolds and cultured for various time points before SEM imaging. *In vivo* studies were performed to evaluate osseointegration of the scaffolds. Scaffolds were implanted for 7 days in tibia osteotomies of Sprague Daley rats. Retrieved tibiae were sectioned and stained with Goldner's Trichrome.

Results/Discussion: hMSCs were cultured for 24 hours and 2 weeks on various scaffolds before imaging by SEM to observe cell number and morphology. As shown in Figure 1, more cells appeared to initially adhere to the biphasic and triphasic scaffolds as compared with PCL alone. Differences in cell numbers were even more striking at 14 days following initial cell seeding. In particular, the cells on the triphasic scaffolds formed a confluent layer by day 14 indicating that cells had proliferated on the scaffolds.

Scaffolds were implanted into rat tibiae to assess osseointegration. A comparison of the *in vivo* response to a 100% PCL scaffold and a PCL/nanoHA composite are shown in Figure 2. Significantly more new bone (stained green) is synthesized around scaffolds that contain nanoHA. In addition to improved osseointegration, nanoindentation measurements showed an increase in both Young's modulus and hardness as nanoHA content in the fiber increased. However, further studies are needed to determine whether the observed increase in cell number *in vitro* and greater bone synthesis *in vivo* is due primarily to increased chemical cues or to increased scaffold stiffness.

Conclusions: Osseointegration of electrospun PCL scaffolds is improved by incorporation of nanoHA (*in vivo* studies of the collagen-containing scaffolds are in progress). Future tests include a study to assess the ability of these materials to induce differentiation of hMSCs along an osteoblastic lineage.

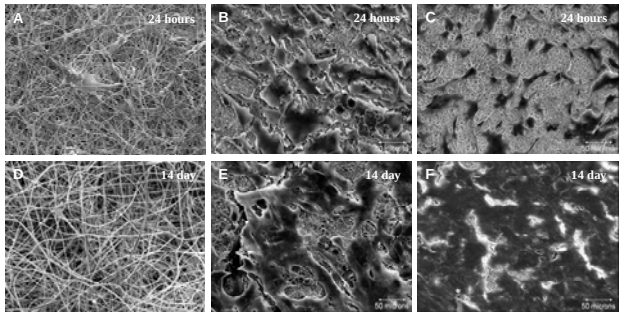

Figure 1. SEM images of hMSCs cultured on nanofibrous scaffolds for 1 or 14 days. (a) and (d) are 100% PCL. (b) and (e) are a composite of 80% PCL, 20% nanoHA. (c) and (f) are a triphasic composite of 50% PCL, 35% nanoHA, and 15% collagen I. The triphasic scaffold produced the highest cell number after 2 week culture.

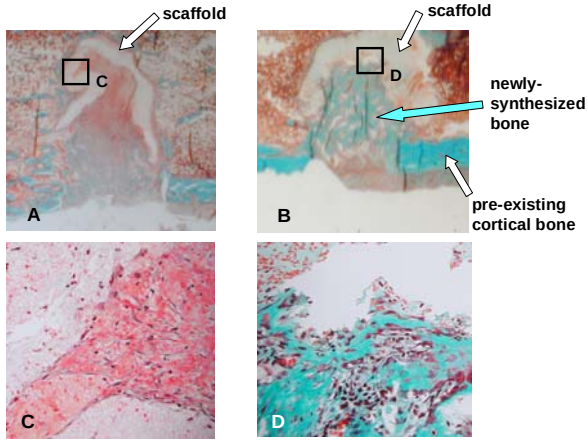

Figure 2. In vivo response to nanofibrous scaffolds placed in rat tibiae. Staining by Goldner's trichrome shows that PCL/nanoHA composites induced more new bone formation than PCL alone (green staining is indicative of mineralized bone). (A) and (C) represent a 100% PCL scaffold; (B) and (D) represent an 80%PCL/20% HA composite. (A) and (B) are imaged at low magnification, while (C) and (D) are higher magnification images of the regions indicated.

References:

1. Catledge SA, *et al*. J Biomed Mater Res A. Submitted 2006.

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