Improved Marrow Stromal Cell Adhesion and Proliferation on Micro/Nano Electrospun Poly(e-caprolactone) Scaffolds

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Statement of Purpose: Synthetic bone scaffolds are continually developed to address the needs of patients undergoing joint-replacement [1], tumor resection and endoprosthetic implantation, maxillofacial repair, and skeletal trauma healing [2]. Techniques such as manipulating scaffold architecture, incorporating biochemical signals, and altering the scaffold chemistry have driven progress towards more highly-engineered scaffolds. A common approach to evaluating these scaffolds has been to use secondary-derived cell lines or passaged primary stem cells. It is more physiologically relevant to use primary stem cells, but passaging may affect cell differentiation, and has been shown to shorten telomeres. Herein, the effects of scaffold architecture on phenotypic behaviors were investigated by the use of electrospun poly(ε-caprolactone) fibers. Bone marrow stromal cells (MSCs) isolated from Wistar rats were seeded directly onto scaffolds in order to more closely mimic the *in vivo* cell community. The short-term (seven day) response of the MSC population was evaluated in terms of viability, adhesion and spreading, colony formation, and cell morphology. After seven days, osteogenic media was used and the cells were evaluated for three weeks in terms of osteoblastic indicators such as calcium deposition, alkaline phosphatase (ALP) production, calcium phosphate formation, and cell morphology. In the short term, micro- and nano-featured scaffold architecture enhanced MSC adhesion and proliferation, and differences of cell migration and colonization were visibly apparent between all scaffolds. After differentiation, scaffolds with nanofibrous (NF) architecture supported a greater level of phenotypic behavior in the cell population after 3 weeks, based on levels of calcium and ALP production, but matrix vesicle attachment was substantially different between control and structured scaffolds.

Methods: Electrospinning produced scaffolds with either purely nanofibers (NF) or microspheres embedded in a nanofibrous matrix (MN). Scaffolds were characterized in terms of their architectural feature sizes with a field-emission scanning electron microscope (SEM). Bone marrow stromal cells were isolated from adolescent Wistar rats and seeded directly onto scaffolds in MSC media. At 1, 4, and 7 days, Live/Dead™ fluorescence staining, an MTT cell viability assay, and SEM examination were performed in triplicate. After 7 days, MSC media was replaced with osteogenic media. After 1, 2, and 3 weeks in the osteogenic media, calcium and ALP were quantified with calorimetric assays and visualized with calcium and phosphate staining. Cells were also examined under SEM and electron-dispersive X-ray spectroscopy (EDS).

Results: Featured scaffolds showed elevated MTT levels over smooth PCL samples, which coincided with greater cell numbers, cell coverage, and colony formation over the course of seven days (Figure 1A, 1B, 1C). Cells on MN scaffolds demonstrated a preference for colonizing on architectural features which were not present in the NF scaffolds, so cells colonized earlier on NF scaffolds than on MN scaffolds.

After differentiation, cells on NF scaffolds had clearly infiltrated the interior of the scaffolds while cells on MN scaffolds remained on the surface. Calcium phosphates were visible and mapped out with EDS (Figure 1D, 1E, 1F), while calorimetric assay showed substantially greater production of ALP on NF scaffolds after the full three week period.

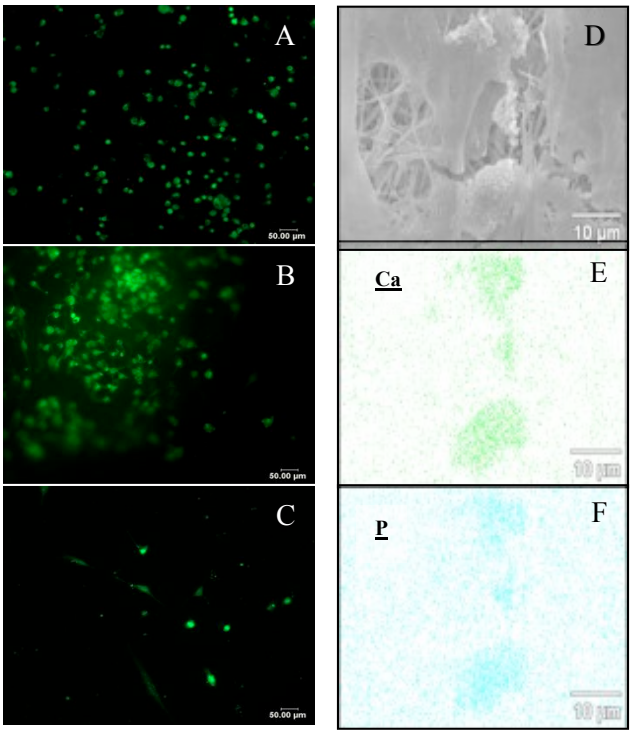

Figure 0 - Live/Dead(TM) images at Day 4 on (A) MN, (B) NF, (C) smooth PCL. SEM (D) and EDS of Calcium (E), and Phosophorus (F) on NF scaffolds

Conclusions: Scaffold architectural features affect the migration and colonization of the marrow stromal cell population in the days following cell seeding. Featured scaffolds demonstrated enhanced cellular activity in this short term. After differentiation, mineralization could be observed on all via the calcium assay as well as EDS. High levels of ALP assay after three weeks suggest that the NF scaffolds were the most osteoconductive.

References:

1. Langer & Vacanti. (1993) *Science*
2. Ishaug *et al*. (1997) *J. Biom. Mat. Res. A*