A Novel, Calcium Phosphate Electrospun Composite Promotes the Osteogenic Differentiation of Stem Cells

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Statement of Purpose: The two most commonly investigated materials for bone repair are β-tricalcium phosphate (β-TCP) ($Ca_3(PO4)_2$) and synthetic hydroxyapatite (HA) ($Ca_{10}(PO4)_6(OH)_2$) since they are biocompatible, osteoconductive, and bioactive [1]. Clinically, however, ceramics have had limited use because of their brittleness and difficulty in shaping [2]. Therefore, biodegradable polymer/bioceramic composites have been sought as an alternative form to using calcium phosphates alone. In this study, composites consisting of varying weight percentages of 20/80 HA/TCP and poly (ε-caprolactone) (PCL) were fabricated using the electrospinning technique. Solvent and solvent combinations were evaluated to form scaffolds with a maximum concentration of ceramic and uniform dispersion. The fabricated electrospun composites were evaluated for bone bioactivity, i.e. the ability to form an apatite on the material's surface. The osteogenic differentiation of human mesenchymal stem cells on these scaffolds was also studied.

Methods: *Scaffold Fabrication:* Electrospun composites of PCL and 20/80 HA/TCP (Composite-MC and Composite-MC+DMF) were fabricated using two different procedures as detailed in a previous study [3]. Composite-MC mats contained a bimodal distribution of fiber diameters with nanofibers in between larger, micron-sized fibers (approx. 28.5 μm) with an average pore size of 79.6 ± 67 microns; whereas, Composite-MC+DMF fibers had uniform fiber diameters (approx. 2.5 μm) with an average pore size of 7.0 ± 4.2 microns.

Immersion Studies: The bioactivity of the scaffolds was evaluated by immersing the scaffolds in simulated body fluid (SBF) for 28 days. The immersed scaffolds were characterized using SEM-EDXA, XRD and FTIR.

RT-PCR studies: Human mesenchymal stem cells (MSCs) were seeded onto scaffolds and cultured in standard growth media or osteogenic induction media for up to 28 days. Comparisons were made with cells seeded on unfilled PCL, 20/80 HA/TCP (Block), and polystyrene culture plate. The gene expression for osteogenic markers of Runx2, Type I collagen and Osteopontin was determined by real-time RT-PCR. Sox-2 gene, which is a transcription factor expressed at high levels in undifferentiated MSCs and pluripotent stem cells was also evaluated.

Results and Discussion: Electrospun composites fabricated using different solvents resulted in varying fiber diameters and pore sizes. The ceramic was distributed throughout the cross-section and surface of the fibers of the mats. Based on our previous characterization results, 30% ceramic weight composites were selected for immersion and cell studies.

Bioactivity Studies: The unfilled PCL electrospun scaffolds did not show any visible apatite formation after immersing in SBF for 28 days whereas, the composites had a visible apatite on the fibers (figure 1). There was a well-developed, globular apatite on the Composite-MC mats whereas the apatite on the Composite-MC+DMF mat was sparse. The SEM-EDXA, FTIR and XRD data confirmed the globules were apatite. SEM-EDXA results showed that the Ca/P ratio of the apatite on Composite-MC was 1.96 whereas the apatite formed on the Composite-MC+DMF was only 1.38. Cross sections of the scaffolds were also examined for the presence of the ceramic inside the fibers. There was no ceramic in the Composite-MC fibers after immersion suggesting the ceramic inside the fibers reacted with the SBF and thus, demonstrating more bioactive properties/reactivity.

RT-PCR Studies: Cells on all materials expressed Runx2, Type I collagen and Osteopontin genes at Day 14. However, Sox2 gene was not expressed, suggesting all materials promoted differentiation of MSCs on day 14 (Figure 2). Cells expressed the highest level of Runx2 and type I collagen on the Composite-MC mats in both standard growth media and osteogenic media.

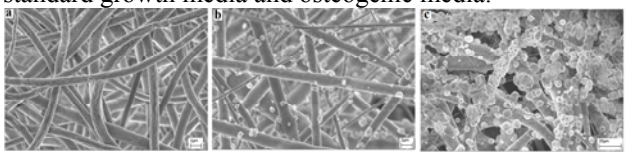

Figure 1: SEM micrographs of electrospun PCL (a) Composite-MC+DMF (b) and Composite-MC (c) immersed in SBF for 28 days

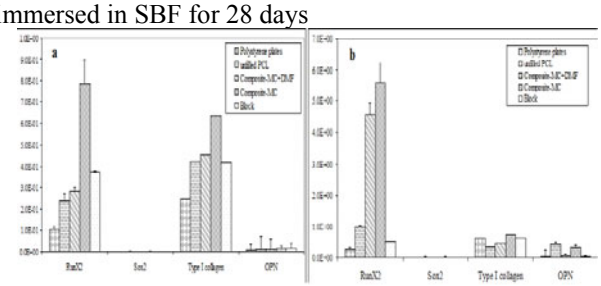

Figure 2: Gene expression data of MSCs in growth media (a) and induction media (b)

Conclusion: Electrospun composites fabricated using different solvents resulted in varying fiber diameters and pore sizes which resulted in varying bioactivity properties and osteogenic activity. Composite-MC demonstrated more favorable properties, suggesting that the novel composite scaffold with larger pore sizes and novel architecture will prove to be a promising scaffold for bone tissue engineering.

References:

1. Z. Zhang. *Oral science International*. 2005; 85-95
2. M. Wang. *Biomaterials* 2003; 24:2133-51
3. A. Patlolla. *Acta Biomaterialia*. 2009; in press.

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