# A Novel, Composite Scaffold for Stem Cell Induced Bone Regeneration

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**Statement of Purpose:** Calcium phosphate ceramics are routinely investigated as bone graft substitutes. The two most commonly used bioceramics 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 20/80HA/TCP and poly (ε-caprolactone) (PCL) were fabricated using the electrospinning technique. The 20/80 HA/TCP composition was chosen as the ceramic component because of previous reports of greater bone tissue formation in comparison to HA or TCP alone [3]. Solvent and solvent combinations were evaluated to form scaffolds with a maximum concentration of ceramic and uniform dispersion to achieve improved bioactivity [4]. Human mesencymal stem cells (hMSCs), derived from adult bone marrow, were evaluated on the composites for viability, growth and osteogenic differentiation over time.

**Methods:**

*Electrospinning:* PCL (Sigma Aldrich, 80,000 MW), nano-HA and nano-β-TCP (avg. size=100nm, Berkeley Advanced Biomaterials, Inc.) were used for fabricating the electrospun scaffolds. For the electrospinning process, PCL was dissolved in either methylene chloride (Composite-MC) or a combination of methylene chloride (80%) and dimethylformamide (20%) (Composite-MC+ DMF).

*Characterization:* The morphology and element analysis of the electrospun mats were studied using scanning electron microscopy (SEM) and SEM-energy dispersive x-ray analysis (SEM-EDX). The pore size of electrospun mats was determined using capillary flow analysis. The porosity was determined from their density values. The tensile properties of the mats were determined using Instron 3342 tensile tester. The amount of ceramic and any residual solvent in the electrospun mat was determined by thermogravimetric analysis (TGA). The crystallization behavior and glass transition of the composites was determined using differential scanning calorimetric (DSC) and thermally stimulated current (TSC) respectively.

*In Vitro Cell Studies*: The cell viability, proliferation, and differentiation of hMSCs on the composite electrospun scaffolds were studied using the XTT cell viability assay kit (Biotium Inc.), picogreen assay (Molecular Probes) and alkaline phosphatase assay (ALP, Sigma), respectively. Cell morphology was observed using SEM.

**Results:** The composite-MC mat consisted of web-like nanofibers in between micron-sized fibers whereas the composite-MC+DMF mat had a uniform fiber size (Figure 1). Fiber diameters, pore sizes and porosity were significantly higher in composite-MC as compared to composite-MC+DMF. SEM-EDX spectrum results confirmed the presence of calcium and phosphorous in both the composites. The TGA data confirmed that the amount of ceramic present in the composites.

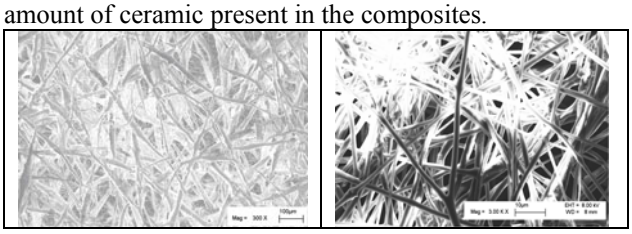

**Figure 1**: SEM micrographs of electrospun Composite-MC (left) and Composite- MC+DMF (right)

| | Tg -I (°C) | Tg-II (°C) | $1/t_{1/2}$-I ($min^{-1}$) | $1/t_{1/2}$-II ($min^{-1}$) | Tc -I (%) | Tc-II (%) |
|---|---|---|---|---|---|---|
| 0% | -53.7 | -54.0 | 0.80 | 0.79 | 44.1% | 41.2% |
| 10% | -52.0 | -52.8 | 0.46 | 0.56 | 44.1% | 42.5% |
| 15% | ---- | -51.0 | ---- | 0.52 | ---- | ---- |
| 20% | -49.8 | -47.5 | 0.43 | 0.50 | 44.9% | 42.5% |
| 25% | ---- | -44.0 | ---- | 0.44 | ---- | ---- |
| 30% | -43.4 | -47.6 | 0.41 | 0.46 | 46.8% | 44.0% |
| 40% | -44.2 | -52.8 | .46 | 0.47 | 46.0% | 44.1% |
| 50% | -54.4 | -53.8 | .54 | 0.49 | 45.0% | 43.2% |

**Table 1:** Glass Transition, Crystallization Rate, and Crystallinity of Electrospun Composites

The crystallization temperature (Tc) increased (lower under cooling) with the addition of the ceramic indicating that the nano ceramics acted as a nucleating agent. This effect was seen more in the composite-MC mats as compared to composite-MC+DMF mats. The glass transition results and crystallization rate results suggest that there is an interaction between the polymer and ceramic (Table 1). Mechanical tests demonstrated higher tensile strength in composite-MC+DMF over composite MC. Cell viability and proliferation studies showed that the scaffolds supported cell growth. The cell differentiation studies in standard growth media, i.e. without osteoinductive factors, demonstrated that the ALP activity was significantly higher on the composites fabricated using composite-MC at day 11 ($p<0.05$) as compared to PCL and composite-MC+DMF. The cell morphology results demonstrated that there was matrix deposition and mineralization on both composites.

**Conclusion:** Electrospun composites were successfully fabricated using different solvents resulting in varying fiber diameters and pore sizes. MSCs on composite-MC demonstrated more favorable results. This suggests that the novel composite scaffold with larger pore sizes, high crystallinity and novel architecture will prove to be a promising scaffold for bone tissue engineering.

**References:**

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