Oleic acid delivered by nanofibers increases bone matrix formation by differentiated marrow stromal cells

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Statement of Purpose: Bone tissue replacement technology is currently hindered by the limited availability of autograft tissue and by the limited capacity to successfully integrate autograft or xenograft constructs *in vivo*(1). Synthetic scaffolds for bone regeneration must address both scalability/reproducibility as well as biological considerations such as tissue integration and regeneration. Electrospinning is a process in which consistent polymer fibers can be fabricated to produce porous, three-dimensional constructs in a repeatable manner. By incorporating charged molecules such as oleic acid (OLA) in the polymer, fiber diameters may be reduced to a nanoscale without losing consistency(2). OLA is an organosoluble fatty acid that has been shown to increase osteoblast phenotypic behaviors in serum-free conditions(3). This study aimed to determine whether inclusion of OLA in polycaprolactone (PCL) would significantly alter nanoarchitecture of the scaffolds, and its release from nanofibers would enhance bone matrix deposition by marrow stromal cells in osteogenic media.

Methods: PCL was dissolved in 3:1 v/v chloroform:methanol at 12% w/w concentration. OLA was added to the solution at either 1% or 5% of the polymer weight. For negative controls, OLA was leached out by soaking in methanol for 24 h. The solution was electrospun to produce continuous nanofibers, and the nanofibers' diameters were characterized using scanning electron microscopy (SEM) (Figure 1).

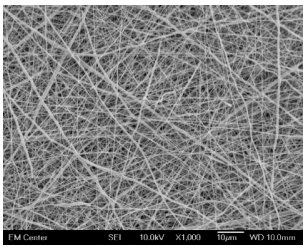

Figure 1 - SEM of PCL nanofibers with 5% OLA. Mag = 1000x, Scale bar = 1 μm

Thermogravimetric analysis (TGA) and digital scanning calorimetry (DSC) were used to analyze the scaffold crystallinity and thermal degradation characteristics. Cells were harvested from the bone marrow of 80-85 wk old Wistar rats and were cultured onto scaffolds. Metabolic activity was measured after 1 and 4 days of culture with an MTT assay, and live cells were imaged after 1, 4 and 7 days of culture with calcein AM staining. After 7 days, cells were differentiated with dexamethasone, glycerol-2-phosphate, and ascorbic acid. Alkaline phosphatase activity (ALP) and calcium deposition on scaffolds were measured colorimetrically up to 3 weeks of culture. After 3 weeks, osteopontin (OP) and osteocalcin (OC) were imaged with immunofluorescent staining and expression levels of both proteins were measured with polymerase chain reaction.

Results and Discussion: Nanofiber scaffolds were found to have insignificant differences in fiber diameter and crystallinity with changes in OLA content. After 7 days of culture, MTT values and coverage for cells on OLA scaffolds were lower than on OLA leached scaffolds. However, at each time point, cells on 5% OLA scaffolds showed enhanced ALP activity (Figure 2) and calcium deposition (Figure 3), whereas the OLA leached scaffolds showed slightly greater osteoconductive characteristics. After 3 weeks, both OP and OC were also expressed in greater amounts on 5% OLA scaffolds.

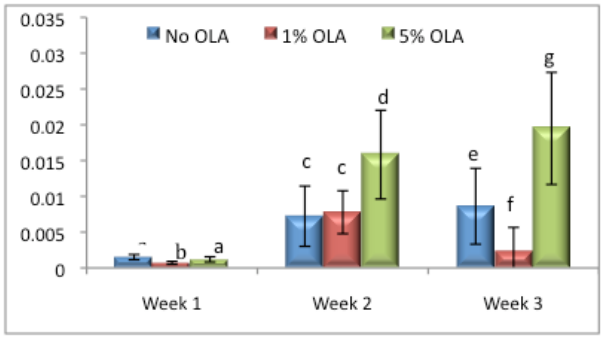

Figure 1 - ALP activity for three weeks of culture, measured by colorimetric assay. Letters indicate statistical grouping ($p \leq 0.05$)

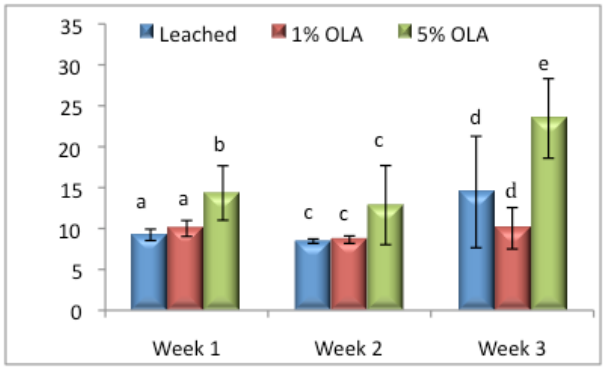

Figure 3 - Calcium deposition onto nanofiber scaffolds, measured by colorimetric assay. Letters indicate statistical grouping ($p \leq 0.05$)

Conclusions: Our results show that OLA in small concentrations in nanofiber scaffolds has a nominal effect on scaffold characteristics. However, its effects on MSCs through the course study were significant by decreasing cell coverage and metabolic activity in maintenance media, followed enhancing bone matrix production in osteogenic media. Further studies are now conducted to characterize the release profile of OLA from nanofiber scaffolds and examining the mechanism of action of OLA on MSCs.

References:

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