Enhanced MSC Activation and Regulation on Poly(ε-Caprolactone) Nanowire Surfaces

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Statement of Purpose: Concerns over utilizing autogenous cancellous bone grafts (such as donor site morbidity, increased surgical time/complication rate, and restricted availability) as the gold standard treatment for critical-sized defects in bone have motivated the development of a wide variety of sophisticated synthetic bone scaffolds in recent years. In this work, a novel solvent-free template synthesis technique was utilized to fabricate poly(ε-caprolactone) (PCL) nanowire surfaces as a building block for the development of 3-D bone scaffolds. Bone marrow-derived mesenchymal stem cells (MSCs) were used to characterize the short and long term *in vitro* biocompatibility and cellular response to these surfaces. A 4-week study in rats was conducted to assess *in vivo* biocompatibility as well. Short term *in vitro* studies revealed that PCL nanowire surfaces enhanced MSC response in terms of survivability, viability, cytoskeleton changes, and morphology as compared with control surfaces (smooth PCL and tissue culture polystyrene). In long term *in vitro* studies, nanowire surfaces induced a rapid cellular production of bone extracellular matrix (ECM) by differentiated MSCs as indicated by accelerated calcium phosphate mineralization, and osteocalcin (OC) and osteopontin (OPN) production. *In vivo* studies and histological analysis confirmed that nanowire surfaces are biocompatible. Biodegradation of PCL nanowire surfaces was also investigated and reported on briefly. This work presents a simple technique for solvent-free fabrication and bioactive molecule encapsulation of biocompatible, biodegradable 3-D bone scaffold components and warrants further investigation.

Methods: A simple, solvent-free template synthesis protocol was followed to fabricate PCL nanowire surfaces. To assess the short term response of MSCs to PCL nanowire surfaces, cell survivability, adhesion, morphology, viability, and cytoskeleton reorganization was determined after 1, 4, and 7 days of culture using standard live/dead kits, MTT-based assays, and immunofluorescence. Based on favourable short term results, long term in vitro analyses were also conducted. MSCs seeded on nanowire and control surfaces were provided with differentiation media for up to 4 weeks and assayed for differentiation and ECM production. Alkaline phosphatase (ALP), Calcium-phosphate mineralization, and bone-specific ECM proteins such as osteocalcin and osteopontin were detected using standard colorimetric and immunofluorescence techniques. Finally, PCL nanowire surfaces were tested for *in vivo* biocompatibility. A 4 week *in vivo* study was conducted in rats using ASTM standard F-763-04 and strictly following all NIH Institutional Animal Care and Use Committee (IACUC) guidelines and regulations.

Results: The simple template synthesis technique utilized was capable of repeatability fabricating uniform arrays of distinctive PCL nanowires. **Fig. 1** displays an SEM image of the surface. Regular microchannels formed which is likely due to surface tension effects during membrane dissolution.

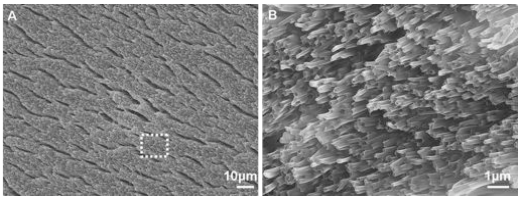

Fig. 1*: SEM images of nanowire surface at 10 keV, at 750X (A), and 8500X (B)*

Live/Dead assays, MTT-based viability tests, and cytoskeleton staining (data not shown) indicated that PCL nanowire surfaces provided a favorable substrate for cell-cell communication, spreading, and migration compared with control surfaces. **Fig. 2** displays live/dead and viability plots for MSCs seeded on nanowire surfaces after 4 days of culture.

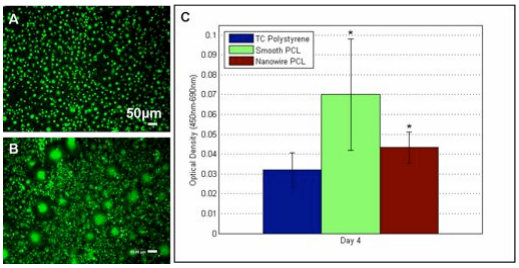

Fig. 2*: Live/Dead for Smooth(A) and nanowire (B) surfaces. Viability (c) was comparable between nanowire and control surfaces.*

Long term *in vitro* studies indicated that MSCs seeded on nanowire surfaces had increased levels of ECM production and mineralization. **Fig. 3** displays enhanced osteocalcin (OC) and osteopontin (OPN) expression on nanowire surfaces after 4 weeks in culture.

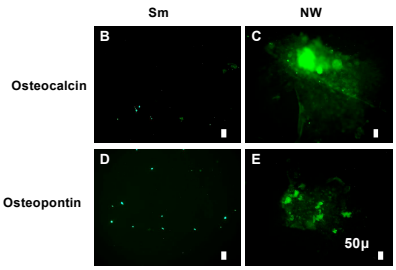

Fig. 3*: Immunofluorescence preparations for OC and OPN on smooth (B,D) and nanowire (C,D) surfaces after 4 weeks in culture.*

In vivo studies indicated that PCL nanowire surfaces were biocompatible and did not initiate chronic inflammation (data not shown).

Conclusion: This work demonstrates that uniform arrays of substrate-bound polymeric nanowires are easily fabricated using a novel template synthesis technique. PCL nanowire surfaces provided a unique substrate for enhanced short and long term functionality and phenotypic behavior in MSCs. Additionally, PCL nanowires displayed excellent biocompatibility as indicated in short term *in vivo* studies. Results of this study suggest that these surfaces warrant further investigation into the development of 3-D bone tissue engineering scaffolds using polymeric nanowire surfaces as the primary building block.

Reference:

Porter JR., Henson A., Popat KC., Biodegradable Poly(ε-caprolactone) Nanowires for Bone Tissue Engineering Applications. Accepted in Biomaterials.