Osteogenic differentiation of mesenchymal stem cells on demineralized and devitalized biodegradable polymer and extracellular matrix hybrid constructs

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Statement of Purpose: A substantial clinical need continues to drive the development of biomaterial-based approaches to promote repair of large bone defects arising from various etiologies. Although graft materials such as allogeneic bone and dimeralized bone matrix (DBM) are widely used in the clinic, these materials undergo devitalization (removal of the living cellular component) and sterilization processes prior to application, which have been shown to reduce the osteogenic and osteoinductive properties of the graft material relative to autologous bone. Increasing effort has been invested in the development of cell-generated extracellular matrix (ECM)-coated constructs to promote bone regeneration, and these constructs have been shown to contain major extracellular matrix components of bone and to promote the osteogenic differentiation of mesenchymal stem cells (MSCs) *in vitro*. However, **t**he devitalization and demineralization techniques of cell-generated ECM coated constructs generally have been adapted from the processing of bone grafts and tissues, and there has been little investigation of the effects of various devitalization methods on the composition of the cell-generated ECM coatings. Accordingly, we investigated whether these modified bone graft processing techniques affected the retention of the ECM components and the osteogenic differentiation of MSCs cultured on MSC-generated ECM coated constructs.

Methods: Hybrid constructs were generated by seeding osteogenically pre-differentiated rat MSCs onto electrospun poly(ε-caprolactone) (PCL) fiber meshes (8 mm diameter, ~1 mm thick; ~10 μm average fiber diameter) and culturing in osteogenic medium for 12 or 16 days within a flow perfusion bioreactor (flow rate of 0.7 mL/min) to create an ECM coating. The resulting constructs were then either devitalized (using a freeze-thaw or a detergent (Triton X-100) technique), devitalized and demineralized, or left untreated (ethylene oxide (EO) sterilization only), and subsequently characterized for DNA, glycosaminoglycan (GAG), collagen, and calcium content using established biochemical assays. The osteogenicity of each construct was investigated by culturing MSCs on the hybrid constructs within a flow perfusion bioreactor (flow rate of 0.7 mL/min) for 4, 8 and 12 days in osteogenic medium followed by biochemical assays for alkaline phosphatase activity and calcium content, which are standard markers for osteogenic differentiation. Scanning electron microscopy (SEM) and histological analysis were conducted using standard methods. Statistical significance between all constructs was determined using Tukey's Honestly Significant Differences test with 95% confidence interval using JMP 9 software (SAS Institute).

Results: The characterization of the constructs demonstrated that all of the devitalization and demineralization techniques tested were successful in removing cells and calcium-bearing minerals. Specifically, the amount of DNA present in the untreated constructs (EO sterilization only) was significantly higher than in any of the respective processed constructs and negligible levels of calcium were observed for constructs receiving demineralization treatment. No significant difference in the amounts of GAGs, collagen, and calcium was measured in each of the treated constructs when compared to the respective untreated constructs. Of all the processing techniques examined, histological analysis demonstrated that the freeze-thaw method contained the thickest coating of GAGs, collagen, and calcium within the constructs. In addition, the loss of minerals from the ECM coated constructs via the demineralization and devitalization process resulted in a reduced osteogenicity of the constructs. Moreover, each of the devitalization and demineralization techniques generated void spaces in the ECM coating, which generally increased with the number of processing steps (Figure 1).

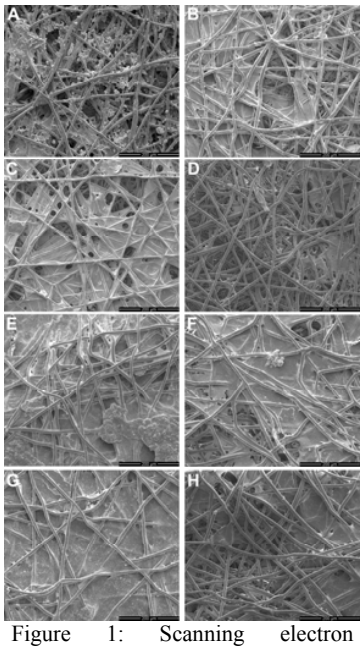

Figure 1: Scanning electron micrographs of processed constructs at 250X magnification. A) Ethylene Oxide (EO) Day 12, B) Freeze-Thaw (FT) Day 12, C) Triton X-100 Treatment (Tri) Day 12, D) Freeze-Thaw and Demineralization (dM) Day 12, E) EO Day 16, F) FT Day 16, G) Tri Day 16, H) dM Day 16.

Conclusions: This study explored the effect of devitalization and demineralization techniques on the retention of ECM components within cell-generated ECM coated constructs and the osteogenic differentiation of MSCs seeded onto these constructs. Overall, the methods applied in the processing of cell-generated ECM coated constructs were found to impact the composition and associated osteogenicity of the constructs.

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