Bone regeneration on nano-fibrous reverse solid freeform fabricated poly(L-lactic acid) scaffolds

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Statement of Purpose: Tissue engineering is an interdisciplinary field that endeavors to develop biological substitutes that restore, maintain or improve tissue function. One such method involves introducing cells and/or tissue-inducing factors to a biodegradable scaffold, where the neo-tissue can regenerate eventually replacing the volume of the degrading scaffold. Since most primary organ cells are believed to be anchorage-dependent and require specific environments for growth, the success of tissue engineering relies greatly on the development of suitable scaffold systems for both *in vitro* tissue culture and subsequent *in vivo* neo-tissue formation. Nano-fibrous (NF) scaffolds are used in an attempt to better mimic collagen in the natural extra-cellular matrix (ECM) and could potentially provide better environment for tissue formation in tissue engineering systems. By combining the phase separation of poly(L-lactic acid) (PLLA) solutions with reverse solid free-form (SFF) fabrication, 3-D NF scaffolds with complex geometries are formed which allow for precise control of internal pore size and structure, as well as external scaffold shape including architectures generated from computed-tomography scans. *In vitro* cell cultivation experiments show improved proliferation, differentiation, and mineralization on NF scaffolds when compared to solid-walled (SW) scaffolds.

Methods: Molds were designed using Rhinoceros or Mimics V8.1 software and then printed layer-by-layer using a Modelmaker II. A solution of PLLA in 4:1 (v/v) dioxane:methanol was stirred at 60°C until homogeneous and then cast into the mold and phase separated overnight at –20°C for NF scaffolds. The solvent was extracted by immersing the composite in cold ethanol (–20°C) for 1 d and in ice-cold water for 1 d. Similarly, the SW scaffolds where cast and phase separated and then lyophilized at –5 to –10°C to remove dioxane crystallites. The mold was dissolved with 37°C cyclohexane and the scaffolds washed in 37°C ethanol and water then freeze-dried. MC3T3 E1(sub 26) cells were then seeded and cultured on each scaffolds in osteogenic culture conditions for up to 6 weeks[1]. At 2 and 6 weeks, osteocalcin (OCN) and Collagen type I (COL) expression were measured using ABI Prism 7500 real-time PCR system. The mineralization was quantified using the *o*-cresolphthanlien complexone method and the mineral distribution was examined using Von Kossa staining after 6 weeks of culture.

Results / Discussion: Over the course of the culture period, the NF scaffolds express higher levels of OCN than the SW scaffolds (Figure 1a). This indicates the NF scaffolds create a more favorable environment than the SW scaffolds for differentiation. The decreased collagen

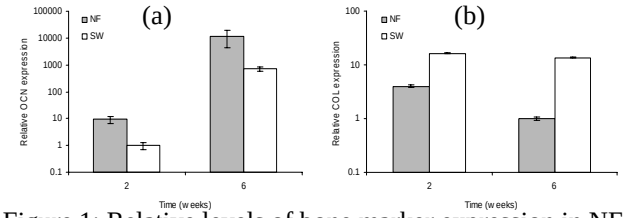

Figure 1: Relative levels of bone marker expression in NF and SW scaffolds (a)osteocalcin (b) collagen, type I

expression on the NF scaffolds compared to the SW scaffolds (Figure 1b) suggests that the cells may interact with the NF scaffolds in a manner similar to the collagen of the natural ECM.

After 6 weeks of culture, the cells in the NF scaffolds have a healthier appearance and the tissue is more organized than in the SW scaffolds. Beyond that, NF scaffolds have increased approximately 80% more mineralized content, which is more evenly distributed through the scaffolds, than the SW ones (Figure 2). This indicates that NF scaffolds provide a more favorable environment for tissue formation and mineralization.

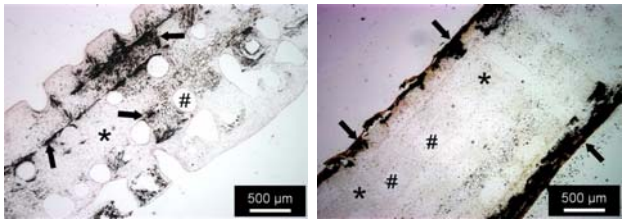

Figure 2: Von Kossa silver nitrate staining of scaffolds after 6 weeks of culture (a) NF scaffold, (b) SW scaffold.

Conclusion: By combining polymer phase separation with SFF, we have created a NF architecture capable of being produced with SFF manufacturing techniques which promotes bone tissue regeneration. In this study we have demonstrated increased differentiation and mineralization on NF scaffolds and the flexibility of the fabrication method to form complex forms. This technique holds great promise to create ideal scaffolds for bone tissue engineering.

Reference:

1. Ma, PX. Zhang, R. Xiao, G. Franceschi, R. J Biomed Mater Res. 2001; 54: 284-293

Acknowledgements: The authors thank Materialise for providing use of their Mimics software. This research was supported by the University of Michigan Nanomaterials Initiative, NIH (DE 14755, DE 15384: PXM), the Training Program in Organogenesis (NIH T32 HD07505, VJC), administered by the Center for Organogenesis of the University of Michigan, and a National Science Foundation Graduate Student Fellowship (LAS).