Optimization of Mesenchymal Stem Cells Performance on Biomimetic Ligament Scaffolds for ACL Regeneration

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Statement of Purpose: The anterior cruciate ligament (ACL) is the most commonly injured ligament of human knee. However, the regeneration of ACL remains a critical challenge in clinic due to its intrinsically poor healing ability and lack of vascularization[1]. In response to this challenge, we have previously developed a biomimetic ligament scaffold (BLS) which provides mechanical support of ACL using a novel 3D braiding technology[2]. Mesenchymal stem cells (MSCs) derived from bone marrow represent an attractive cell source for ligament tissue regeneration due to their multipotency and clinical availability. However, the performance of MSC on BLS has not been explored partially due to the unfavorable surface properties of these scaffolds. Herein, we focused on the optimization of MSC cellular behaviors on BLS via a series of surface treatment strategies. Our objective is to develop BLS with improved cellular performance that can be translated into clinic.

Methods: Poly-L-lactic acid (PLLA) microscale fibers were braided into BLS using 3D braiding technique developed in our lab. Three different kinds of surface modifications on BLS were conducted: 1) BLS with plasma treatment for different times (5, 10, and 15 min); 2) BLS adsorbed with three different amounts of fibronectin (Fn) (10, 25, 50 μg/mL); and 3) BLS first treated by plasma (PT) for 5, 10, and 15 min followed by Fn coating for 2 h. Rabbit MSCs (RMSCs) purchased from vendor were expanded and then seeded onto BLS with different surface modification at 8×10^4 cells/scaffold. The impact of surface treatment on RMSC adhesion was evaluated by CellTiter-Blue assay, SEM, immunocytochemistry (ICC). The proliferation of RMSCs on BLS was monitored at different times up to 21 days. The ECM deposition on the PLLA microfibers was observed with SEM. Gene expression of tenogenic markers was performed using qPCR to evaluate the impact of surface treatment on RMSC differentiation towards tenocyte lineage.

Results: BLS was composted of PLLA fiber bundles with average fiber diameter ~20 μm. Fn was successfully immobilized onto the BLS due to the high affinity of Fn to PLLA surface. PT on PLLA decreased the contact angle from 79 to 49 degree. Both methods showed their potential in optimizing BLS surface properties. RMSC adhesion was significantly improved by various surface treatments: RMSC on untreated PLLA BLS remained rounded shape (Fig. 1-A), indicating poor cell adhesion. RMSCs on scaffolds treated with Fn, PT, and Fn/PT all demonstrated more spread out morphology and the number of adhered cells at 8h also increased compared with untreated PLLA (Fig. 1-A). After three weeks of culture, RMSCs on BLS have fully covered the PLLA microfiber surface and cells also aligned along the direction of the fibers. Large amount of fibrous ECM

deposition was observed, suggesting the cells started to secret collagen that assembled into nanofibers. ICC staining shows similar results where RMSCs populated the whole scaffold as well as covered the entire surface of the fibers (Fig. 1-B). Fig. 1-C shows Fn treatment were also found to be able to promote cell proliferation for up to 2 weeks (Fig. 1-C). Similarly, plasma treatment combined with Fn coating significantly increased RMSC growth after compared to untreated PLLA scaffolds (Fig. 1-D).
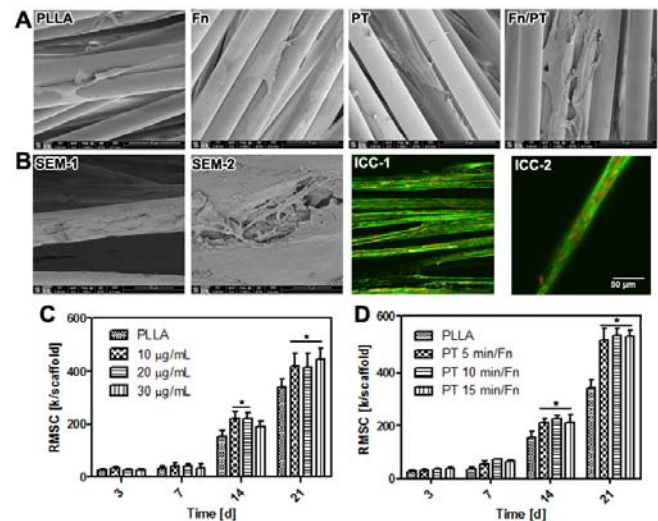

Fig1. Short and long term performance of RMSC on BLS with varying surface treatment: A) RMSC adhesion on BLSs: PLLA: Untreated BLS braided with PLLA microfibers; Fn: PLLA BLS coated with fibronectin PT: PLLA BLS treated with plasma for 10 min; Fn/PT: PLLA BLS treated with plasma and coated with Fn. B) SEM-1 and -2 show RMSC aligned on microfiber after 3 wks of culture and showed fibrous ECM deposition, ICC staining shows full coverage of aligned RMSC on PLLA microfibers. C) Effect of Fn coating of BLS on RMSC proliferation. D) Effect of plasma treatment combined with Fn coating on RMSC proliferation on BLS.

Conclusions: These results demonstrate the feasibility to improve cellular performance of BLS using various surface treatment strategies. These data provide critical information to improve ACL scaffold design into clinical applications. The resultant BLSs with improved surface properties might serve as optimal stem cell delivery carrier to facilitate the tissue engineered ligament regeneration. Future studies will focus on the evaluation of ACL regeneration efficacy in vivo using autologous MSC combined with biomimetic ligament scaffolds with optimized surface properties.

References: [1] C. T. Laurencin, J. W. Freeman, *Biomaterials* **2005**, *26*, 7530. [2] J. A. Cooper, J. S. Sahota, W. J. Gorum, J. Carter, S. B. Doty, C. T. Laurencin, *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 3049.