Mechanical Stimulation Induced Fibroblastic Differentiation of *h*MSCs on Nanofiber Scaffolds

Subramony, SD; Dargis, BR; Castillo, M; Azeloglu, EU; Tracey, MS; and Lu, HH
Columbia University, Department of Biomedical Engineering, New York, NY, hl2052@columbia.edu

Introduction: The anterior cruciate ligament (ACL) is the most frequently injured knee ligament[1]. Due to complications associated with biological grafts used for ACL reconstruction, there is a pressing clinical need for alternative grafts. Our long term goal is to develop a functional and integrative scaffold for ACL tissue engineering. Polylactide-*co*-glycolide (PLGA)[2] based scaffolds have been researched for ACL tissue engineering due to their relevant mechanical properties, and have also been reported to promote fibroblast growth and biosynthesis[3]. Building upon these successes, our approach utilizes biomimetic PLGA nanofiber scaffolds for directing human mesenchymal stem cell (hMSC)-mediated ligament regeneration. Specifically, the **objective of this study** is to determine the effect of mechanical stimulation on the differentiation of hMSCs seeded on aligned PLGA nanofiber scaffolds in a custom-designed loading bioreactor. **It is hypothesized** that cyclic tensile loading of hMSCs on nanofibers will guide their differentiation into ligament fibroblast-like cells and stimulate the production of a ligament-like matrix.

Methods: *Scaffold fabrication & Cell Culture:* Aligned PLGA (85:15, Lakeshore) nanofiber scaffolds (fiber diameter=615±152nm, elastic modulus=341±30 MPa) were formed via electrospinning[4]. Commercially obtained hMSCs (Lonza) were seeded on nanofiber scaffolds (30,000 cells/cm^2). *Study Design:* The experimental group was subjected to 1% uniaxial tensile strain for 90 minutes twice daily in a custom bioreactor. Control samples were cultured in identical bioreactors without loading. Samples were collected after 1, 7 and 14 days of culture. *End-Point Analyses:* Cell attachment was assessed using the Live/Dead assay. The effect of loading on cellular alignment was quantified (n=3/group) using circular statistical analysis[5]. Total DNA (n=5) was determined by Picogreen assay, while collagen synthesis (n=5) was measured via the Sircol assay. Collagen distribution was visualized by Picrosirius Red staining (n=2). The expression (n=3) of ACL fibroblast markers (collagen I, III, fibronectin, tenascin C) and integrins α1, αV, α5, and β1 was examined by RT-PCR (expression normalized to GAPDH). *Statistical Analysis:* ANOVA and the Tukey-Kramer *post-hoc* test were used (*p<.05).

Results: ***Cell Attachment/Growth:*** hMSCs exhibited an elongated spindle-like morphology, and were oriented along the long axis of the nanofiber (Fig. 1A). Cell number was significantly greater in the loaded group at week 1 (Fig 2A). Circular statistical analysis revealed that cell alignment was guided by the nanofiber scaffold and followed the loading direction, although no difference was observed between the loaded and unloaded groups (Fig. 1B). While collagen production was similar between groups, greater matrix penetration was observed in the loaded group (Fig 2B). ***hMSC Differentiation:*** The expression of relevant ACL fibroblast markers was significantly upregulated under applied tensile loading. Collagen I, III, fibronectin, and tenascin C expression all increased by day 14 ($p<0.05$) for hMSCs on loaded scaffolds versus unloaded controls. Additionally, the expression of integrins α2, α5 and β1 increased significantly with loading by day 14 (Fig. 3).

Discussion: The results of this study demonstrate that nanofiber scaffolds coupled with cyclic tensile loading promote the differentiation of hMSCs into ligament fibroblast-like cells. Interestingly, cell attachment was predominantly guided by nanofiber organization resulting in no significant difference in alignment between loaded and unloaded groups. Similar to the study by Altman *et al.*[6], we observed the upregulation of phenotypic fibroblastic markers with dynamic tensile loading, coupled with the upregulation of key integrins which have been reported by Hannafin *et al.* to become over-expressed in ligament fibroblasts in response to tensile loading[7]. The upregulation of these integrins, which serve to bind collagen and fibronectin, provides insight into the mechano-transduction pathways of hMSCs on nanofibers and will be investigated further. Overall the results of this study demonstrate the potential of nanofiber scaffolds coupled with physical stimulation to promote stem cell-mediated ACL regeneration. Future studies will focus on scaffold optimization and *in vivo* evaluation.

References: *1)Glickson et al., AAOS Bulletin, 2004. 2)Lu et al., Biomaterials, 2005. 3)Moffat et al., Tissue Eng., 2008. 4)Reneker et al., 1996. 5)Costa et al., Tissue Eng., 2003. 6)Altman et al., FASEB J., 2002. 7)Hannafin et al, J. Orthop. Surg, 2005..*
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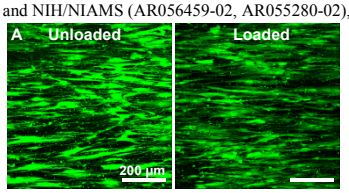

B

Day 14 (n=3)	*Mean Angle ± Angular Deviation*
Unloaded Cells	4.59±8.64
Unloaded Fibers	3.46±5.87
Loaded Cells	6.72±9.18
Loaded Fibers	4.41±7.77

Figure 1: Cell Attachment. A) Cell morphology for the loaded and unloaded groups (Day 14, 20x). B) Mean vector angle (MA: -90° ≤ θ ≤ 90°)± angular deviation (AD:0-40.5°). 0° indicates horizontal orientation.

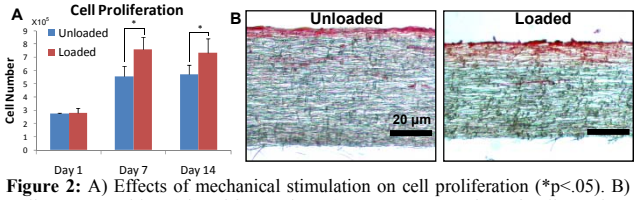

Figure 2: A) Effects of mechanical stimulation on cell proliferation (*p<.05). B) Collagen Deposition (Picrosirius Red, 32x). Deeper penetration of collagen into loaded scaffolds versus unloaded scaffolds. Note differences in scaffold thickness (20.7 ± 3.2 % decrease) due to the application of cyclic tensile loading.

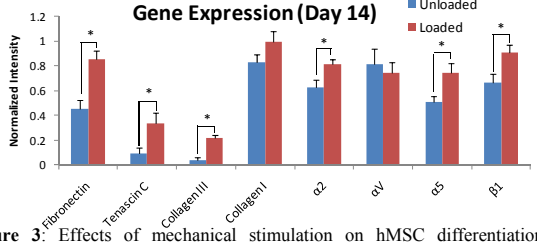

Figure 3: Effects of mechanical stimulation on hMSC differentiation. The expression of fibronectin, tenascin C, collagen III as well as the α2, β1 and α5 integrins was upregulated by day 14 (*p<.05).