Tendon Tissue Engineering Using the Human Umbilical Vein and Mesenchymal Stem Cells

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Statement of Purpose: The goal of this study was to investigate the potential of using the Human Umbilical Vein (HUV) seeded with mesenchymal stem cells (MSCs) as a tendon tissue replacement model.

Materials and Methods: Fresh human umbilical cords were procured from Norman Regional Hospital in Norman, OK. Using an automated lathe HUVs were dissected out of the umbilical cords to a uniform wall thickness of 0.75 mm and decellularized in 1% sodium dodecyl sulfate. Bone marrow Mesenchymal Stem Cells (MSCs) were mixed with type I collagen and inserted in the central portion of the HUV at a seeding density of 1 million cells/ml. A bioreactor, specifically designed for this project, was used to mechanically stimulate 3 constructs simultaneously at 2% strain for a period of 1 hour/day and a frequency of 0.0167 Hz. The seeded constructs were divided into three groups as follows: (Group 1) cultured in 100-mm diameter well plates; (Group 2) cultured in the bioreactor without any mechanical stimulation; and (Group 3) cultured in the bioreactor with 1 hour of stimulation per day.

Results and Discussion: An increase in cell number was measured for all groups after 2 weeks of culture, however the increase was at least 8 fold higher for stimulated samples of group 3 (Figure 1-a). Stretching may affect surface receptors and/or stretch activated ion channels[1] leading to an increase in proliferation rates. Moreover, cyclic loading potentially enhances mass transport through the HUV thus mitigating potentially existing mass transport limitations of nutrients. qRT-PCR conducted 2 weeks post culture showed an upregulation in the expression of both collagen I (4 fold) and collagen III (3 fold) in stretched constructs of group 3 compared to other groups.

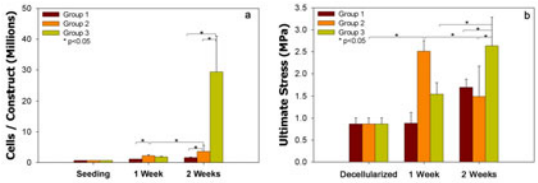

Figure 1

All cellular groups had improved mechanical properties 1 and 2 weeks post culture compared with the decellularized HUV (Figure 1-b). Stretching improved the mechanical properties of the seeded scaffolds resulting in significantly stronger (156%) and stiffer (109%) constructs compared to un-stretched samples after 2 weeks of culture.

Microscopically, unstretched samples (Figure 2-a) had random orientation of fibers with an average deviation from the axial direction of 34.57 ± 48.16°. Stimulated constructs, on the other hand, showed parallel orientation of collagen fibers (Figure 2-b) with an average deviation of 1.69±17.6° away from the axial axis. A closer view at the longitudinal sections showed rounded nuclei for cells in the static cultures (Figure 2-c) with a shape factor of 0.9 compared to spindle shaped nuclei for the dynamic cultures with a shape factor of 0.7 (Figure 2-d). Thus, stretching aligned collagen fibers parallel to each other and gave the cells a spindle shape mimicking the morphology of native tendons[2]. Stretching of the nuclei of the MSCs could be an indication of their differentiation to tenocytes[3].

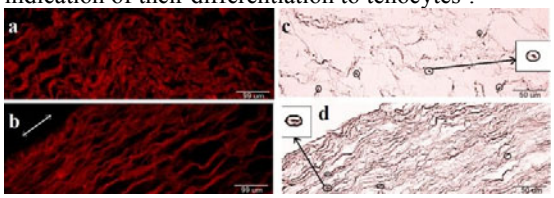

Figure 2

In an attempt to optimize the seeding density in the HUV, different seeding densities were investigated: Low Seeding Density (LSD=1 million cells/ml), Medium Seeding Density (MSD=5 million cells/ml), and High Seeding Density (HSD=10 million cells/ml). For the MSD, cellularity within the constructs increased more than 2 folds 1 week post culture (Figure 3-a). These results were consistent with proliferation values within HUVs seeded with the LSD. With similar proliferation rates, MSD stimulated HUVs had an ultimate stress value of 4.1 ±0.5 MPa, almost 4 folds higher than stretched LSD constructs (Figure 3-b). However, proliferation ceased after 2 weeks of culture, and constructs had poorer mechanical properties with seemingly thinner HUV walls and degraded ECM. Histologic images revealed lysed cell bodies 2 weeks post culture for MSD and HSD constructs. It appears that at high seeding densities not enough glucose diffused through the HUV to support cell survival and proliferation, and metabolic biproducts, such as lactic acid, could not diffuse out of the HUV, creating a toxic acidic environment in the scaffold. The optimal seeding density should be less than 5 million cells/ml, and the limiting seeding density was calculated based on glucose consumption rates to be 3 million cells/ml.

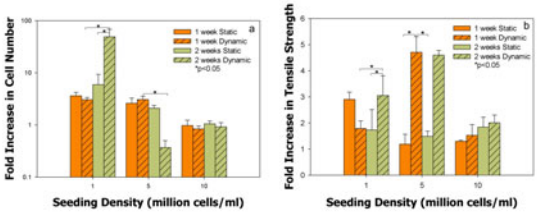

Figure 3

Conclusions:

Results have shown the ability of MSCs to proliferate and migrate deep into the HUV scaffold when seeded at 1 million cells/ml and subjected to 1 hour of cyclic stretching/day. Mechanically stimulated constructs showed a tendon like appearance 2 weeks post culture with ultimate tensile strength values only 1 order of magnitude lower than human tendons. Higher seeding densities resulted in weaker and less cellular constructs after two weeks of culture. The optimal seeding density was found to be 3 million cells/ml.

References:

1. Butler DL. Annu Rev Biomed Eng. 2004;6:303-29
2. Doroski DM. Biomaterials. 2007;28:187-202
3. Altman GH. FASEB J. 2002;16:270-2