The Human Umbilical Vein as a Scaffold for Tendon Tissue Engineering

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Statement of Purpose: More than 32 million acute and chronic injuries occur to tendons and ligaments every year in the United States.[1] For critical injuries, grafts are often utilized due to poor healing since the tendon has limited integrated vasculature and lymphatic systems. However, traditional grafts in some instances can cause donor site morbidity, graft rejection, and incomplete healing.[2] Replacement tendons utilizing tissue engineering can reduce or avoid these complications. The research presented here utilizes a novel tissue engineering scaffold, the human umbilical vein (HUV) seeded with mesenchymal stem cells (MSCs) which is cultured long term in a custom-made bioreactor to develop the construct.[3] The HUV is used since it possesses mechanical strength more similar to tendons than traditionally used hydrogels while still supporting excellent cell growth and development.[2,3]

Methods: The HUVs are extracted from locally obtained umbilical cords. The umbilical cords are frozen to -80°C and then the HUV is extracted utilizing a computer-controlled lathe. To remove any residual cells and genetic material, the HUV is subjected to sodium dodecyl sulfate, ethanol, and peracetic acid washes. Afterwards, the HUV is cut open flat to allow for MSC seeding (1.8 million cells/scaffold). Seeding efficiency tests were performed to determine whether seeding was more efficient on the luminal or abluminal surface of the HUV. Once that was determined, the tissue is then placed into the bioreactor which is then cultured for 7, 14, 21, and 28 days. During this culture, a 1 hour/day cyclical stretching is applied of 2% strain at 1 cycle/min frequency. Constructs are then harvested from the bioreactor and analyzed for tensile strength, cellularity, histological/morphological structure, and gene expression (through quantitative RT-PCR). Results are presented as mean ± SD and a sample size of at least three is used in all measurements.

Results: The first step was to determine the best surface to seed cells onto. Seeding on the abluminal, or tissue, side resulted in a yield of 60% seeding efficiency while the luminal, or smooth muscle, side only had a seeding efficiency of 25%. This can be attributed to the abundance of glycosaminoglycans and proteoglycans found in the umbilical cord tissue and the rougher surface to facilitate better cell attachment, compared to the smooth, endothelial side. Cellularity analysis of the tissue showed a typical exponential growth through day 28. After 28 days, the tissue possessed 26 ± 6 million cells, a 14 fold increase. However, this was similar in number to previous studies after only 14 days[3] The difference between that study and this study was previously the HUV remained in a cylindrical shape and cells were seeded in the interior, protected from circulating media flow and resulting fluid shear. In this study, cells were exposed, and some cells may have been lost due to detachment from the fluid shear.

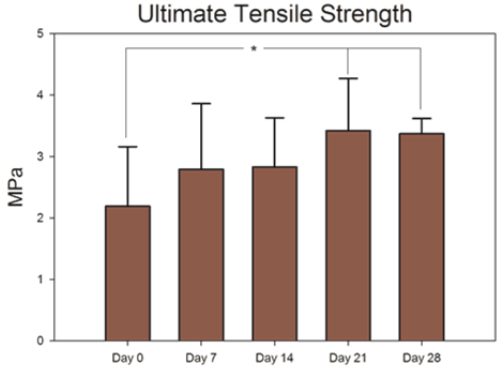

Figure 1: Mechanical Strength of Tissue Construct

However, this exposure to the fluid limited transport issues that were seen in the cylindrical configuration. However, even with less net cellularity, the tensile strength increased 65% from 2.1 ± 1.0 MPa to 3.4 ± 0.3 MPa throughout the culture (figure 1). This increase is most like due to increased tissue deposition and realignment of this new and existing tissue by the cells. Histological images showed that as culture times progress from 0 to 28 days there was continual improvements of the quality of the tissue. Dececullarization of the scaffold results in random and finer fibrils within the matrix. However, after only 7 days, alignment of these fibers in the direction of stretching occurs, a characteristic of tendons. From 14 to 28 days, fibril diameters and alignment increased, until it resembled dense connective tissue after 28 days. Histology also showed that cells initially seeded on the surface of the scaffold did penetrate throughout.

Finally gene expression was also measured compared to non-stimulated controls. It was seen after 21 days that many extracellular matrix components and protein genes related to tendon development and maturation were upregulated 2 to 10 fold, indicating the cells progressing towards a tendon like lineage after 3 weeks. These included collagen type I, collagen type III, biglcyan, and tenomodulin among others.

Conclusions: This study demonstrated that the MSC-seeded HUV is a viable scaffold when cultured as a flat sheet in addition to previous research which utilized it as a cylinder. Cellularity and mechanical strengths increased through the culture. In addition, histological images and gene expression showed that the MSCs and the construct itself was becoming more tendon-like. Future studies should be conducted where initial culture occurs in the cylindrical HUV form to initially protect against fluid shear effects of the bioreactor and then opened up for long-term cultures. By doing this, the properties of the artificial tissue may increase even further.

References: [1] Schoen, D.C. Orthop Nurs, 2005:24:304-307 [2] Józsa, L.G. Human tendons : anatomy, physiology, and pathology. 1997. [3] Issa R. Tissue Eng, 2011:17:1479-1487