Mechanostimulated Wharton's Jelly Stem Cells Seeded into Human Umbilical Veins for Tendon Tissue Engineering

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Statement of Purpose: Previous research has demonstrated the viability of the human umbilical vein (HUV) to serve as a scaffold for tendon tissue engineering applications[1]. Separate research has shown that Wharton's jelly stem cells (WJSCs) can differentiate into bone, cartilage, adipose, muscle, and neural cells[2]. WJSCs have been shown to meet the definition of pluripotency as set forth by the International Society for Cellular Therapy[2]. In our study, a mechanical stimulator previously shown to promote proliferation and tenocytic differentiation in MSCs seeded on HUVs was utilized on WJSCs seeded into the same scaffold material with the aim of investigating the effects of duration and frequency of mechanostimulation on tenocytic differentiation[3]. The stimulation profile that resulted in the greatest upregulation of tenocytic marker genes consisted of 2% strain, delivered in a sinusoidal fashion at 0.0167 Hz, 1 hr/day (for 3 days). These results suggest that similar to MSCs, WJSCs undergo tenocytic differentiation when exposed to mechanostimulation. There may be some heretofore unknown stimulation profile that is ideal for inducing tenocytic differentiation of WJSCs.

Methods: Human umbilical cords were separated from full-term placentas, trimmed to 8.5 cm in length, rinsed with deionized water, and mounted through the HUV on stainless steel mandrels (McMaster-Carr, Atlanta, GA) prior to freezing. The mounted cords were frozen to -80 °C at a rate of -2.5 °C/min. An automated lathe was used to separate the HUV from the cord (while frozen) and simultaneously trim HUV wall thickness to 750 µm. The resulting HUV scaffolds were allowed to thaw, inverted (so that lumenal and ablumenal sides were reversed), and then trimmed to 6.5 cm in length prior to being subjected to the decellularization process described elsewhere[3]. Cells were isolated from similarly obtained umbilical cords by explanting Wharton's jelly to sterile tissue culture-treated polystyrene 6-well plates, where cultures were regarded as passage 0. At passage 3, cells were rinsed with PBS, trypsinized (0.25% trypsin-EDTA, Invitrogen, Carlsbad, CA), counted, suspended in a bovine collagen I (Invitrogen, Carlsbad, CA) solution, and injected into the interior void space of the HUV scaffold. Seeded scaffolds were allowed to incubate for 40 minutes for collagen cross-linking. Seeded HUVs were then installed in bioreactors of custom design (Fig. 1). The bioreactor administered a 2% strain in a sinusoidal manner. Experiments were conducted in triplicate using the following experimental groups: unstimulated (static) bioreactor (control) (Group 1); 0.0167 Hz, 1 hr/day (Group 2); 0.0333 Hz, 1 hr/day (Group 3); 0.0083 Hz, 1 hr/day (Group 4); 0.0167 Hz, 2 hr/day (Group 5); 0.0167 Hz, 0.5 hr/day (Group 6). Each group was stopped after 3 days. Genes of interest included collagen I, collagen III, scleraxis, tenomodulin, cartilage oligomeric matrix protein (COMP), biglycan, decorin, matrix metalloproteinase-3 (MMP-3), and tenascin-C. Gene expression was assessed using the $\Delta\Delta C_t$ method using glyceraldehydes-3-phosphate dehydrogenase (GAPDH) as the housekeeping gene.

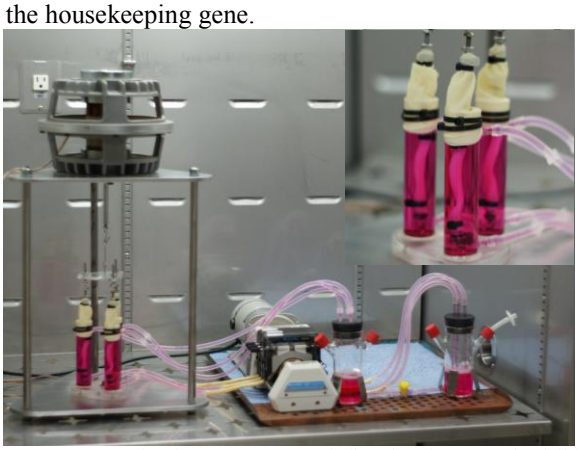

Figure 1: Example Bioreactor Setup. Bi-directional actuator is visible above culture chambers. Media reservoirs and peristaltic pump are also visible. ***Inset:*** Detail of seeded scaffolds anchored in culture chambers

Results: Compared to static controls, gene expression of collagens I and III, tenomodulin, COMP, decorin, and MMP-3 were most highly upregulated in Group 2. Group 2 exhibited a downregulation of tenascin, whereas the other groups either showed modest upregulation or no significant change. Group 5 showed the second-highest upregulation of COMP and MMP3, while showing the greatest downregulation of tenomodulin.

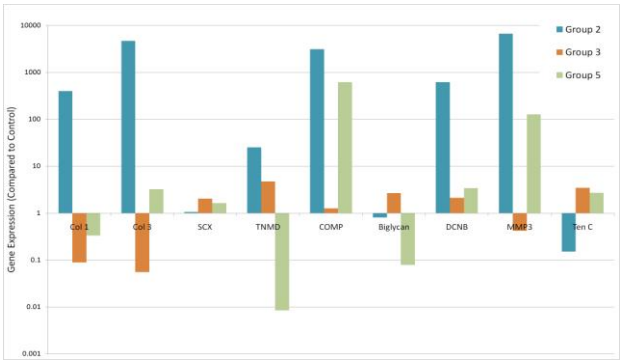

Figure 2: Relative gene expression of select experimental groups

Discussion: Group 2 appears to represent the best-suited stimulation profile for inducing tenocytic differentiation in WJSCs at least for the early stages of culture observed here. Though tenascin-C, which has been linked to development of tendon, cartilage, and bone, is downregulated in this experimental group, the strong upregulation of tenomodulin and COMP are both indicative of a tenocytic lineage. Our results suggest there may be a mechanostimulation regime in which chondrocytic differentiation interferes with the desired tenocytic one (Group 5).

References:

1. Abousleiman, R. I., *Artif. Organs* 32 (9), 735-742, 2008.
2. Shukunami, C., *Biochem Biophys Res Comm* 280, 1323-1327, 2001.
3. Abousleiman, R. I., *Tissue Engineering: Part A* 15 (4), 787-795, 2009.