Engineering Tendons using Adult Stem Cells Seeded in a Mechanostimulated HUV

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Statement of Purpose: More than 32 million injuries occur to tendons and ligaments every year.[1] As treatment, grafts are utilized due to poor native healing of the tendon. However, traditional grafts in some cases can cause donor site morbidity, graft rejection, and incomplete healing. Replacement tendons utilizing tissue engineering can reduce or avoid these complications. To create a better tendon construct, mechanical and chemical stimulation can be utilized to improve its properties.[2] Our research utilizes a novel scaffold, the human umbilical vein (HUV) which as a waste tissue is relatively easy to maintain and also avoids potential xenografts scaffold issues.

Methods: The engineered construct is composed of a decellularized HUV as a scaffold seeded with mesenchymal stem cells (MSCs) in the interior of the cylindrical vein, and cultured in a dynamic bioreactor for up to 14 days. However, previous research indicated that nutrient transport properties decreased as time progressed, due to increased extracellular matrix (ECM) production.[3] Therefore, a new culturing method was developed by cutting the vein and opening it up into a flat sheet. To allow for MSC attachment on the scaffold, the scaffold is seeded statically for 1 day with 1.8 million MSCs. The construct is then cultured dynamically for up to 28 days. Cyclical mechanical stretching was provided for 0.5 hr/day at 2% strain and 0.5 cycle/min. Two experimental groups were investigated: culturing in the bioreactor immediately as a flat sheet, or culturing as a cylinder as was originally done and then opening into a flat sheet after 14 days and allowing for an additional 14 days of culture.

Results: Opening the scaffold up initially and culturing for 28 days in the bioreactor did increase cellularity, ECM quality, and mechanical properties. However, when compared to the construct that was cultured for 14 days in a cylinder and then opening it up into a flat sheet for an additional 14 days, tenocytic gene expression was delayed, mechanical property increases were not as great, and some osteogenic genes were also upregulated. The initially open scaffold did possess 26.0 ± 6.4 million cells after 28 days (a 14.4 fold increase), 72% more than the construct that was only opened after 14 days.

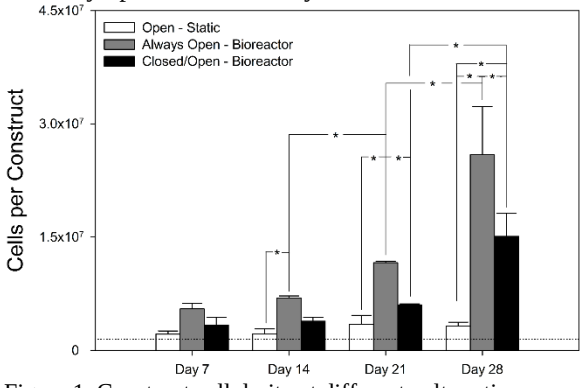

Figure 1. Construct cellularity at different culture times

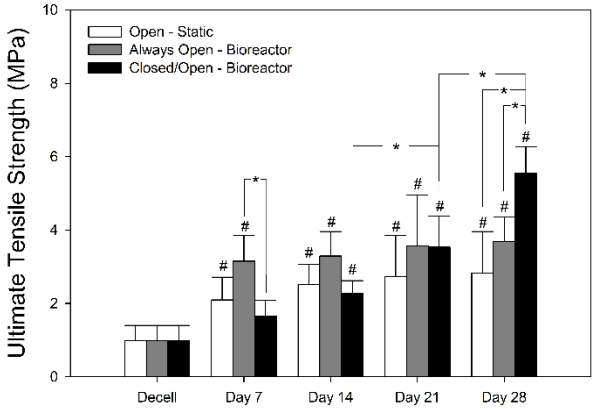

Figure 2. Ultimate tensile strength of constructs at different culture times

However, the initially open scaffold was 50% weaker after 28 days compared to the initially cylindrical construct, which had a 5.6 ± 0.7 MPa ultimate tensile strength. This was reflected in the histological images, while both had dense tissue appearance, the initially open construct was much less aligned in the direction of stretching. In regards to gene expression, the initially cylindrical construct expressed significant upregulation of scleraxis after 14 days. The scleraxis expression decreased at 28 days, while there was an increase in the mature tendon marker, tenomodulin. The initially open scaffold did not show significantly upregulation of scleraxis until day 28, and no upregulation of tenomodulin was seen. However, the initially open scaffold did see significant upregulation of osterix at day 21 and day 28, along with upregulation of osteocalcin at day 28, while the initially cylindrical scaffold did not. Much of this was thought to be due to the presence of shear forces on the cells by the circulating media when the scaffold is opened. Shear forces have been shown to promote cell proliferation and osteoblastic differentiation. The initially open scaffold's cells experience these shear forces immediately and the cells show poorer penetration throughout culture than the cylindrical scaffold, allowing for continued exposure. By the time the cylindrical construct was opened up, cells were mostly within the scaffold wall, isolated from shear forces that would send competing osteoblastic signals to the differentiating MSCs.

Conclusions: This study showed that the HUV/MSC construct is a viable option for tendon tissue engineering. By limiting exposure to fluid shear forces by leaving the construct as a cylinder initially, culture times can be extended by multiple weeks, increasing its properties which can further approach the native tendon such as its tensile strength.

References:

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