Characterization of a Human MSC Population Required for Scaffold-less Construct Fabrication

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Introduction:
Previous work from our lab, has demonstrated successful fabrication of a multiphasic bone-ligament-bone (BLB) graft for ligament repair from sheep bone marrow stromal cells (BMSCs) that were expanded *in vitro* to passage 3, without the use of exogenous or stiff, scaffolding materials[1, 2]. While this process yielded successful ligament for anterior cruciate ligament (ACL) repair, the method is labor intensive, performed in an open cell culture system and not up to fabrication standards sufficient to obtain FDA approval. Thus, the objective of this study was to utilize a commercially available and well-characterized human MSC (hMSC) population as our starting material and evaluate the ability of the hMSC to differentiate directly from BMSSs to tendon and form a 3D construct using a closed bioreactor fabrication process and identify a clinically relevant, regulatory compliant, pre-manufactured starting material for ligament/tendon construct fabrication.

Methods:
The study characterized the biomechanics, morphology and the ligamentous hMSC lineage throughout the ligament fabrication process via gene expression. Briefly, hMSCs (Lonza and Rooster Bio) were seeded onto sterile polystyrene tissue culture plates at a density of 21,000 cells/cm^2. Plates were immediately induced towards bone and ligament linages through the addition of osteogenic and fibrogenic media, respectively. Both hMSCs manufacturers provided extensive characterization of the starting cell populations. At various defined stages of the fabrication process, cells and tissue were harvested for subsequent qPCR analysis at 0, 4, 8, and 12 days. These time points correspond to the initial plating, growth phase, differentiation phase, and 3-D roll-up phase of the self-delamination tissue fabrication process, respectively. At day 12, 3-D constructs were tested for biomechanics and frozen for subsequent morphology via H&E and IHC for collagen content.

Results:
We successfully fabricated three-dimensional bone and ligament constructs utilizing undifferentiated hMSCs using novel BLB bioreactor. At day 12, the mean tangent modulus of the ligament portion of the construct was 12.0 ± 0.2 kPa (Figure 1). No significant differences in the mechanical properties of the BLB were observed between cell lots (n>2 per donor). Morphologically the constructs were similar in appearance to previously described constructs derived from ovine cells. The constructs were composed primarily of Col-1 with viable nuclei present throughout the tissue. Gene expression relative to GAPDH expression was analyzed via quantitative PCR (qPCR) for all tissue samples. Expression of genes typically associated with tendon and bone differentiation was analyzed and compared over time in culture (days 0, 4, 8, and 12). Relative gene expression of tendon differentiation markers Col-1, Ten-C, and Scx were significantly increased at day 8 compared to undifferentiated MSC starting cell populations. While the expression of Col-3 was increased by day 8 (P=0.07), the increased expression was not significant until day 12. The expressions of ligament markers Col-1, Ten-C, and Scx peaked at day 8 before dropping at day 12 to undifferentiated (day 0) expression levels. Additionally, expressions of Runx-2 and ALPL within our ligament constructs were significantly less at days 8 and 12 compared to undifferentiated MSC populations. The down-regulation of these bone-specific markers at day 8 and 12 time points further confirms the induction of the MSCs towards a tendon/ligament lineage.

Discussion:

The results of this study validate the use of our closed bioreactor manufacturing process to engineer 3D tendon and bone constructs starting from a well-characterized commercially available hMSC cell source. Both tendon and bone tissues, fabricated directly from an undifferentiated hMSC population and expanded to ligamentous or osteogenic lineages during the fabrication process, successfully formed 3D BLB constructs. Temporal gene expression confirmed the commitment of human derived constructs toward tendon and bone-like tissues.

Significance:
In summary, the use of well-characterized, undifferentiated, bone marrow derived hMSCs is an acceptable starting cell population for the fabrication of our scaffold-less constructs. As these hMSCs are capable of differentiating towards both ligamentous and osteogenic lineages during the construct formation process, significant time associated with expansion and characterization of a pre-differentiated tendon or bone-like hMSC population can be eliminated. Additionally, sufficient quantities of these well-characterized cells can be readily obtained from FDA compliant companies, making them an ideal input material for our translational manufacturing process

References:
1. Ma, J., et al., *Three-dimensional engineered bone-ligament-bone constructs for anterior cruciate ligament replacement.* Tissue engineering. Part A, 2012. **18**(1-2): p. 103-16.
2. Ma, J., et al., *Morphological and functional characteristics of three-dimensional engineered bone-ligament-bone constructs following implantation.* Journal of biomechanical engineering, 2009. **131**(10): p. 101017.

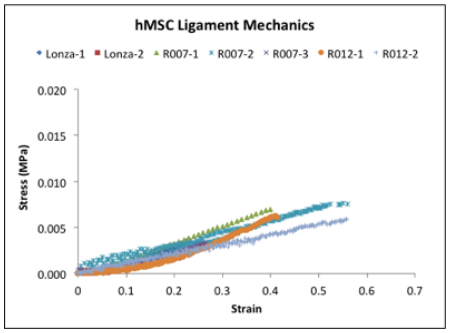

Figure 1. Mechanics of hMSC Ligament Constructs. All tests are shown until failure.

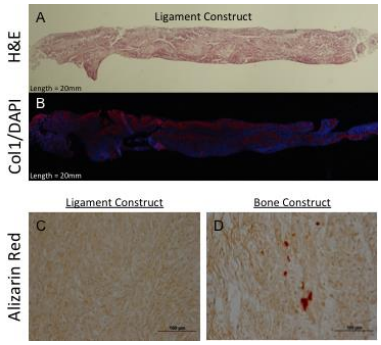

Figure 2. Histology of Human-Derived Constructs A) H&E (X4), B) Collagen-1/DAPI immunostaining (X4), C) Alizarin staining (X40) of ligament constructs, and D) Alizarin staining of bone construct (X40).