Co-isolation of Satellite Cells and Fibro-adipogenic Progenitors from Human Skeletal Muscle

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INTRODUCTION: Satellite cells (SCs) and fibro-adipogenic progenitors (FAPs) are progenitor cell populations found in muscle that are necessary for post-injury regeneration because they form new myofibers and release trophic factors, respectively. Muscle tissue engineering will likely require robust populations of these progenitors, yet SC isolation and expansion are difficult given their scarcity in muscle (<5% of total myonuclei) and limited muscle biopsy size. Hence, simultaneous isolation from human muscle samples is ideal, but unproven in current literature. Here, we investigated a novel cocktail of digestion enzymes to–for the first time–enable dual isolation of significant SCs and FAPs populations from human skeletal muscle.

METHODS: This study was approved by IRB and informed consent was obtained from participants. SCs and FAPs were isolated from surgical remnants obtained from ACL reconstruction surgeries with hamstring autografts (n=14). Dispase and collagenase type I & II cocktail was used to digest muscle and single cells were subsequently sorted into CD56+CD31-CD45- (SC) and CD56-CD31-CD45- (FAP) cell populations using Fluorescence-activated Cell Sorting. These cell pools were then expanded in culture and characterized for lineage-specific markers and differentiation capacity. Statistical analysis was performed on Prism-GraphPad software. Student t-test was used for two-pair comparison while ANOVA followed by Tukey's post hoc test was used for multiple comparisons.

RESULTS SECTION: Post-dissociation, we obtained ~10% SCs and ~36% FAPs, which is at least 2-fold better than reported in current literature. SCs stained positive for Pax7 and retained CD56 expression and myogenic potential after multiple passages, i.e., cell fusion. Simultaneously, FAPs expressed both CD140a and CD140b and differentiated into either fibroblasts or adipocytes upon induction.

DISCUSSION: Overall, this is the first study to demonstrate robust isolation of both SCs and FAPs from the same muscle sample with satellite cell recovery more than 2 times higher than what is reported in the field.

SIGNIFICANCE/CLINICAL RELEVANCE: The high efficiency and co-isolation of SCs and FAPs will enable novel translational and tissue engineering studies for muscle injuries.