Skeletal Muscle Regeneration Utilizing Muscle Derived Stem Cell Augmented Scaffolds: Preliminary Studies to Create an Optimized *in vitro* Construct

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INTRODUCTION: Skeletal muscle loss can result from trauma or oncologic resection, leading to severe cosmetic and functional deficits. Muscle derived stem cells (MDSCs) can be isolated by using a serial preplating technique and have inherent myogenic potential.[1] Since their identification, previous research on MDSCs has mostly focused on *ex-vivo* gene therapy applications aimed at treating muscular dystrophy, and little is known about the optimal population for skeletal muscle tissue engineering.[2, 3] The aim of this study is to determine which preplate population is better suited for muscle regeneration and optimize the *in vitro* conditions for creating an implantable skeletal muscle construct.

MATERAIAL AND METHODS: Murine MDSCs were isolated from transgenic B6 mice expressing red fluorescent protein (RFP) under a ubiquitin C promoter. Proliferation studies of various preplate populations were performed by utilizing bright-field microscopy to determine time to confluence. Myogenic potential was assessed by immunostaining for myosin-heavy chain (MyHC) to evaluate myotube formation. *In vitro* skeletal muscle constructs were created using various concentrations of RFP-expressing MDSCs ($5x10^5$, $1x10^6$, $2x10^6$ cells/construct) seeded onto decellularized muscle scaffolds, and cellular viability and scaffold repopulation were assessed using confocal microscopy. After 14 days in culture, constructs were further analyzed by histology and immunofluorescence.

RESULTS: Preplates 3, 4 and 5 demonstrated faster expansion rates from time of isolation compared to prelates 1, 2 and 6 ($p<0.05$). In terms of their myogenic potential, preplates 3, 4, and 5 had comparable fusion indices (2.53 ± 0.51, 3.22 ± 0.80, 3.10 ± 1.46, $p=0.316$). Confocal imaging of MDSC-seeded construct demonstrated cellular viability at 48 hours and 14 days with progressive concentric scaffold repopulation at all three cellular concentrations. Immunostaining for MyHC demonstrated myotube formation within the constructs after14 days in culture. Further histologic analysis by H&E stain confirmed successful scaffold repopulation and higher cellular density in the constructs seeded with $2x10^6$ cells.

CONCLUSION: The results of this study indicate that preplates 3, 4 and 5 possess high expansion rates and myogenic potentials. Furthermore, MDSCs are capable of successful repopulation of decellularized muscle scaffolds and myotube formation in a 3-dimensional construct. Future experiments will assess the muscle regeneration capability of the MDSC-enriched scaffolds *in vivo*.

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