Engineering 3D Muscle Tissue to Evaluate Cultured Muscle Stem Cell Function

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Introduction

Muscle-related conditions such as sarcopenia and muscular dystrophy affect millions and impair the body's ability to regenerate muscle. Sarcopenia alone impacts up to 50% of individuals over the age of 80 [1]. Understanding the mechanisms of muscle repair may reveal strategies to enhance muscle function, especially in aging populations.

Muscle stem cells (MuSCs) are essential for skeletal muscle regeneration. These cells reside in the muscle stem cell niche and become activated in response to injury or stress, initiating repair through proliferation and differentiation into contractile myofibers. The progression from stem cells to differentiated myofibers can be tracked by measuring expression of markers such as Pax7 to indicate stemness and myosin heavy chain (MHC) to signify differentiation into mature myofibers. In addition to biochemical factors, biophysical cues such as substrate stiffness have been shown to significantly influence MuSC behavior, highlighting the importance of mechanical regulation of cell fate [2].

Our understanding of the mechanisms regulating MuSC mechanotransduction remains limited, in part due to the difficulty of testing stem cell function through in vivo engraftment assays. To overcome this, we are developing an in vitro engraftment assay to probe how substrate stiffness impacts the regenerative potential of cultured MuSCs. We propose that primary MuSCs combined with C2C12 cells, an immortalized mouse myoblast line, in 3D microtissue cultures will serve as a suitable model to probe MuSC function. These platforms will support discovery of strategies to enhance stem cell expansion and enable future studies on mechanical regulation of MuSCs.

Method and Materials

Primary mouse muscle stem cells (MuSCs) were cultured on polyethylene glycol (PEG) based hydrogel substrates. C2C12 cells were cultured in standard growth media. Two microtissue groups were created: (1) pure C2C12 cells and (2) C2C12 with MuSCs cultured on hydrogels. To make the microtissues, we designed a 16 well rigid 3D-printed plastic positive to create a soft polydimethylsiloxane (PDMS) mold based on the published MyoTACTIC 96 well-plate format, featuring posts to anchor the tissue at both ends of each well [3]. Each microtissue contained 450,000 cells suspended in a hydrogel matrix consisting of fibrinogen and Geltrex, which was crosslinked by thrombin. For the microtissues that consisted of both MuSCs and C2C12 cells, a 1:20 ratio of MuSCs:C2C12s was used. After microtissue compaction, myotube differentiation

media was added to each microtissue. After 14 days of differentiation, muscle microtissues were analyzed via immunofluorescence.

Results and Discussion

To evaluate the regenerative potential and functionality of the engineered muscle tissues, we compared the two groups for Pax7 expression and myotube alignment. The pure C2C12 microtissues show functional alignment of sarcomeric alpha-actinin (SAA) and myosin heavy chain (MF-20) positive myotubes (Figure 1,a). This condition also showed low quantities of Pax7 expressing cells (Figure 1,b). This is likely due to the low, but existent, dedifferentiation potential of C2C12 cells to give Pax7+, quiescent-like stem cells. The C2C12 microtissues with MuSCs cultured on hydrogel substrates exhibited substantially higher quantities of Pax7 expressing cells (Figure 1,b), suggesting an ability of the cultured MuSCs to populate the stem cell compartment. As prior work has shown improved engraftment potential of hydrogel-cultured MuSCs in vivo [3], this result suggests that our microtissue model can recapitulate some key features of prior in vivo engraftment assays.

Conclusion

C2C12 cells alone form aligned myotubes in 3D cultures but show a limited ability to populate the stem cell compartment. Co-encapsulation of hydrogel cultured muscle stem cells with C2C12 cells significantly enhances the number of Pax7+ stem-like cells present in the microtissues, demonstrating that this approach may be an efficient means of assaying MuSC function in vitro. These microtissues will serve as platforms to study how mechanical environments influence muscle stem cell function, with an eye towards improving stem cell-targeting therapies in muscle wasting disorders.

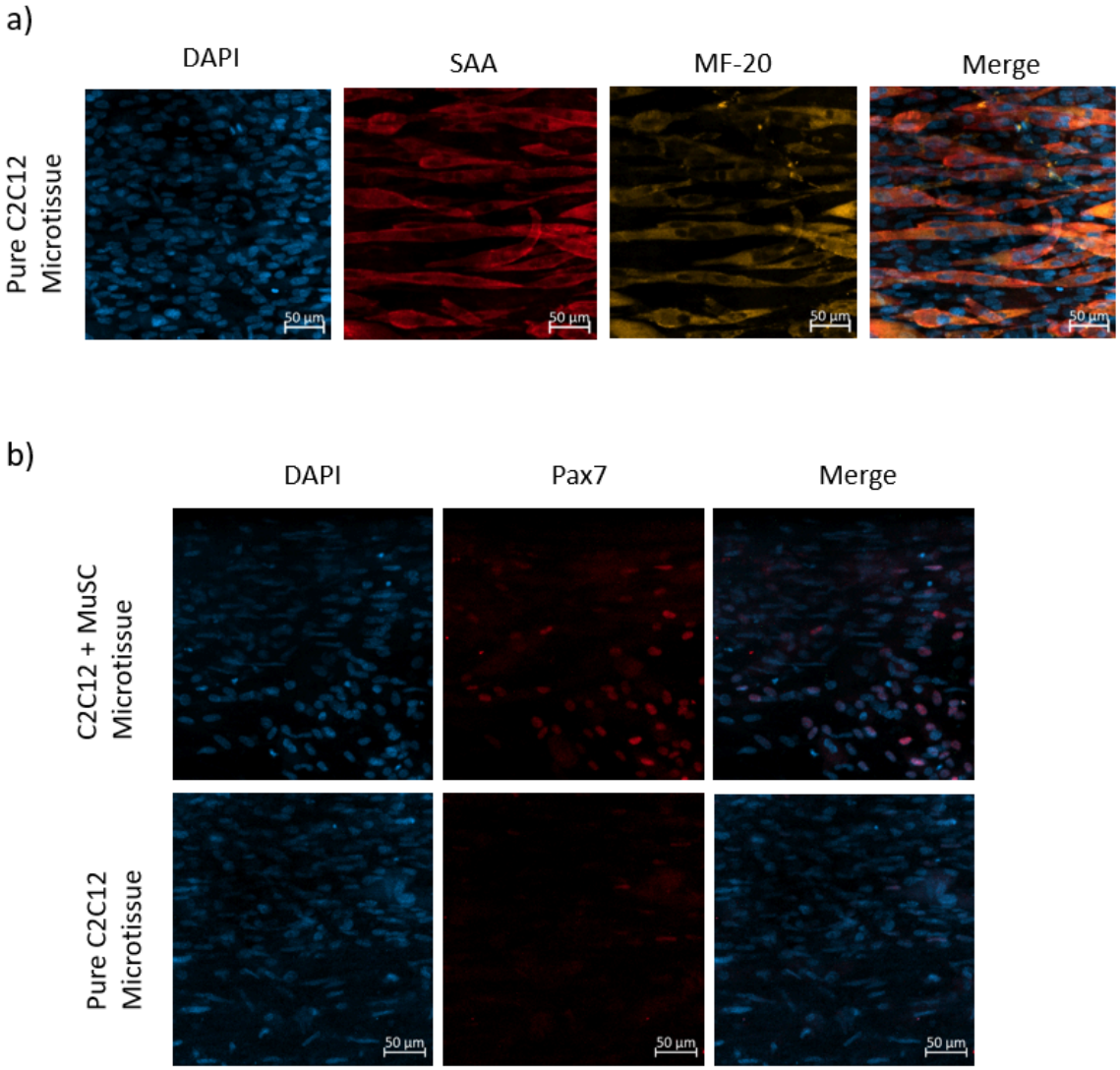

Figure 1. (a) C2C12 microtissues form differentiated, aligned myotubes. (b) The addition of hydrogel-cultured MuSCs enhances the number of Pax7+ cells in the microtissues.

References

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