Functional Enhancement of Bioreactor Assisted Engineered Skeletal Muscle

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Introduction

Tissue engineering of clinically applicable functional muscle tissues is challenging due to the lack of a bioreactor system that would allow for adequate cellular organization and enhanced tissue formation. To bioengineer muscle tissues, single cells expanded in culture are placed on biocompatible scaffolds to form tissue structures. However, single muscle cells seeded on scaffolds usually exhibit multidirectional cellular orientation and fail to self-organize. To overcome this challenge, we developed a tissue bioreactor system that accommodates large muscle tissue which allows for enhanced cellular organization and accelerate tissue formation. We have demonstrated that muscle cell seeded scaffolds exposed to a constant biomechanical stimulation are able to achieve enhanced cellular organization. In this study we investigated whether contractile function could be enhanced by fusing individual muscle cells to form myofibers.

Materials and Methods

The computer-assisted bioreactor system consisted of an actuator mounted on a tissue culture-compatible container which was designed to provide controlled cyclic strain to muscle tissue scaffolds. Primary human skeletal muscle precursor cells were isolated, grown and expanded in culture. The cells were placed in a muscle differentiation medium to induce cell fusion. The cells were seeded onto collagen-based muscle scaffold strips with matrigel coating (1.0 x 0.3 x 0.3 cm^3). The scaffolds containing skeletal muscle cells were placed in the bioreactor system and the controlled cycle strain was programmed to exert ± 10-20% of the cell-seeded scaffold at a frequency of 3 times per minute for the first 5 minutes of every hour. The bioreactor was continuously operated for up to 3 weeks after the initial set up. Non-differentiated muscle cell seeded scaffolds with and without cyclic stimulation served as controls. The muscle cell constructs were assessed for structural and functional parameters with using scanning and transmission electron microscopy, histo- and immunohistochemistry, and physiologic contractile function studies.

Results and Discussion

The differentiated single muscle cells fused and formed multinucleated myofibrils in culture. In contrast, the non-differentiated cells failed to form any myofibrils. The bioreactor stimulated engineered muscle produced viable tissue with appropriate cellular fusion and organization (Fig. 1). Scanning electron microscopy of the bioreactor stimulated muscle tissue showed uniform myofiber attachment of muscle cells on the scaffolds. Histologically, the engineered muscle demonstrated unidirectional orientation and expressed muscle markers.Electrophysiological studies using a patch clamp system showed that the current-voltage relationships were similar between the non-differentiated and differentiated skeletal muscle cells. However, the linear regressions of current-voltage plots before and after application of acetylcholine were significantly different in the differentiated skeletal muscle cells, but not in the non-differentiated skeletal muscle cells (Fig. 2).

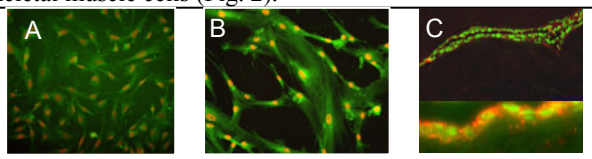

Figure 1. Non-differentiated cells failed to form myofibrils (A). Single differentiated muscle cells fused and formed multinucleated myofibrils in culture (B). The bioreactor stimulated muscle tissue showed myofiber formation on the scaffolds (C).

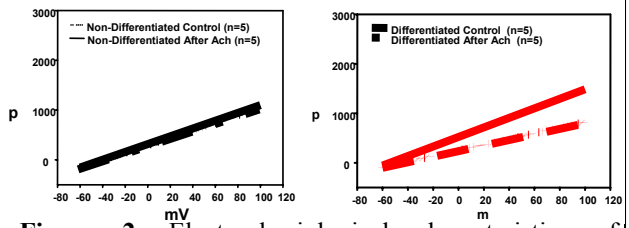

Figure 2. Electrophysiological characteristics of skeletal muscle cells. The linear regressions of current-voltage plots before and after application of acetylcholine were significantly different in skeletal muscle cells with GM (A) and DM (B). Ach: acetylcholine; SKMCs: skeletal muscle cells; GM: growth medium; DM: differentiation.

Conclusions

This study demonstrates that the culture differentiated muscle cells are able to fuse and form multinucleated myofibers. The differentiated muscle cells show significant contractile function. These findings suggest that differentiation of muscle cells prior to the bioreactor stimulation may enhance muscle tissue function in vivo.