‘Fusion of Primary Human Skeletal Muscle Cells within a 3D-Construct’
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INTRODUCTION: To date there have been two approaches to tissue engineer lost or damaged muscle: the in vitro approach to create differentiated muscle tissue constructs for implantation by inducing the fusion of myoblasts to myotubes in 3D culture (Bach et al. 2004) and the in vivo approach injecting muscle-precursor cells into sites of dysfunction – the hope here is that the cells will reorganize spontaneously to form new muscle tissue. The aim of this study was to induce fusion of CD56+ primary human muscle derived cells (PHMDCs) by investigating the effect of increasing cell density and Plastic Compression (PC) (Brown et al. 2005) to create a 3D differentiated muscle tissue construct. Key to this was the need to demonstrate the appearance of myogenin as a marker of myoblast differentiation.

METHODS: PHMDCs were seeded at increasing densities in 3D-collagen gels. The optimal cell density was determined by monitoring the force contraction profile generated by the constructs on a culture force monitor (CFM). To further induce myoblast fusion PC was used to increase cell density and decrease total volume of the construct, to facilitate fusion. RT-PCR was used to detect myogenin, a marker of myoblast differentiation. Finally, TEM was used to identify multinucleated (fused) cells.

RESULTS:

Fig. 1: Force Contraction profiles of Primary Human Skeletal Muscle Cells with changing cell density

The contraction profile of PHMDCs seeded at densities of 4, 6 and 8 million cells/ml (Fig. 1) generated peak forces of 675, 1700 and 930µN respectively over 24 hours. Myogenin expression was identified in constructs at a density of 6 million cells/ml and in the equivalent PC constructs. Multinucleated cells within 3D collagen and PC constructs using TEM were identified (Fig. 2).

Fig. 2: TEM depicting multinucleated cell in a 3D (a) normal and (b) PC collagen construct at 24 hours. Arrows indicate the presence of two nuclei within a single cytoplasm.

DISCUSSION & CONCLUSIONS:
We have established that fusion of PHMDCs within a 3D construct is strongly dependent upon cell density and proximity. The optimal cell density within our defined 3D collagen construct was determined to be 6 million cells/ml. These constructs were then used for PC to further increase cell density and improve mechanical strength. The tissue engineering of a new 3D differentiated muscle tissue construct was verified by the presence of the gene myogenin. This construct will be used as a model of skeletal muscle to investigate and test the effect of mechanical stimulation on muscle cell differentiation, growth and mechanical strength.

REFERENCES:

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