'Fusion of Primary Human Skeletal Muscle Cells within a 3D-Construct'

M.Brady[1,2], R.Brown[1], M. Lewis[2] and V.Mudera[1]

[1] *Tissue Repair and Engineering Centre (TREC), Institute of Orthopaedics, University College London, UK*

[2] Division of Biomaterials and Tissue Engineering, *Eastman Dental Institute, University College London, UK*

INTRODUCTION: To date there have been two approaches to tissue engineer lost or damaged muscle: the i*n 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:

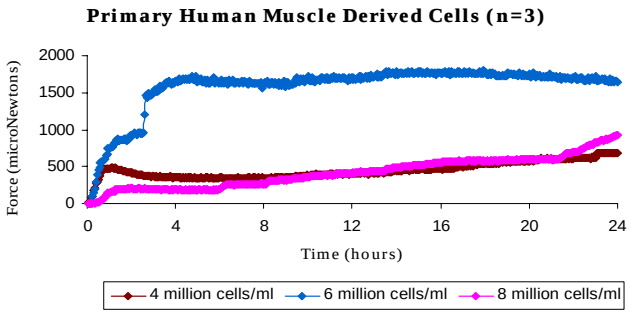

Fig. 1: Force Contraction profiles of Primary Human Skeletal Muscle Cells with changing cell density

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*).

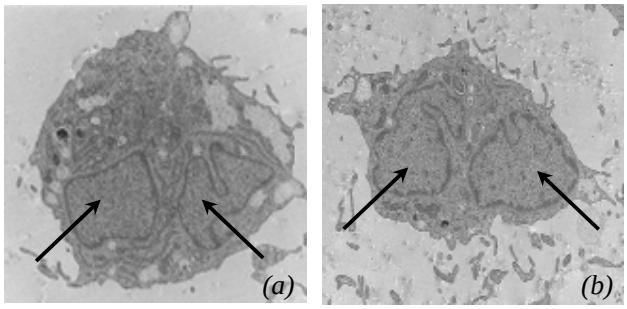

Fig. 2: TEM depicting multinucleated cell in a 3D (a) normal and (b) PC collagen construct at 24 hours. Arrows indicate the presence of two nuclei within a single cytoplasm.

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.

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