Mechanical stimulation of 3D Bio-Engineered Skeletal Muscle

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INTRODUCTION: Skeletal muscle is a highly plastic tissue, responding to exercise and mechanical loading. *In vitro* culture systems have been used to replicate this mechanical stimulus in order to study cellular and molecular adaptations. Previous research using such models has often lacked bio-mimicity, with respect to the *in vitro* culture, the mechanical loading, or both. This has lead to contradictory findings with regards to a variety of molecular outputs. Cell culture matrix and environment (2D or 3D), the type of mechanical loading (uni-axial or multi-axial) and the extent, speed and duration of stretching, are all likely to affect the adaptive responses of the cells and their maturation into functional muscle models. It is therefore necessary to develop a model which has greater physiological relevance if such models are to be used to further understand *in vivo* physiology.

METHODS: 3D collagen based constructs seeded with C2C12 cells (n= 6) were engineered as previously described (Mudera *et al.* 2010). Following 14 days of maturation, the constructs were transferred to an alternative chamber and tethered to the Tensioning Culture Force Monitor (t-CFM) (Fig. 1). The t-CFM is an apparatus whereby programmable regimes of mechanical strain can be applied to the construct by mounting the construct mould to a stepper motor. The mechanical stimulus used was as follows; 7.5% strain, continuous cyclic stretch for 60 minutes. N= 3 constructs were used as static controls. Conditioned media was sampled immediately post stretch for Lactate analysis. Gels were also sampled for RNA extraction. qRT-PCR was performed and gene expression was conducted using the $\Delta\Delta C_T$ method. Statistical analyses were performed using SPSS.

RESULTS: The t-CFM was successfully installed in the laboratory. Different stretch modalities have been programmed for further experimentation, including cyclic and ramp modalities.

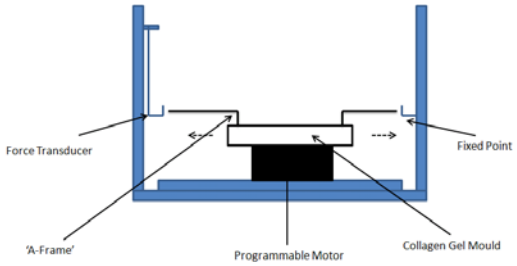

Fig. 1 Schematic diagram of the t-CFM

The stretch protocol used for the current investigation induced significantly higher concentrations of Lactate versus control immediately post stretch (3.17 ± 0.1 mmol.L and 9.8 ± 0.2mmol.L, $p<0.05$). Total RNA concentrations were also significantly higher (316.07 ± 249.21ng/µL) in stretch versus control (121.27 ± 100.07ng/µL, $p<0.05$). Relative expression of Myogenin, a Myogenic Regulatory Factor (MRF) implicated in muscle adaptation increased immediately post stretch versus control (0.087 ± 0.48 and 2.15 ± 1.67, p= 0.13).

DISCUSSION & CONCLUSIONS: Initial stretch experiments have shown acute responses similar with those seen in exercise *in vivo*. These include both classical biochemical markers of responses to exercise (Lactate) and molecular outputs (Myogenic gene expression). Further experimentation within our laboratory aims to specifically identify responses associated with different exercise modalities e.g. resistance and endurance training.

REFERENCES: Mudera V, Smith AS, Brady MA, Lewis MP, (2010), The effect of cell density on the maturation and contractile ability of muscle derived cells in a 3D tissue-engineered skeletal muscle model and determination of the cellular and mechanical stimuli required for the synthesis of a postural phenotype. *J Cell Physiol.* Vol. 225(3):646-53.

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