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MECHANICAL LOADING OF 3-D MUSCLE CONSTRUCTS

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INTRODUCTION: Mechanical conditioning of many tissue engineered construct will be critical, particularly mechano-responsive tissues such as skeletal muscle. It has been shown that application of defined uniaxial loads to 3D constructs through the tensioning- culture force monitor (t-CFM), has been shown to regulate protease expression in fibroblasts (Prajapati et al. 2000). Insulin-like growth factor (IGF-1) is an important growth factor in proliferation and differentiation of skeletal myoblasts (Florini et al. 1996), along with it a recently isolated isoform, mechano-growth factor (MGF) is found to be upregulated in skeletal muscle *in vivo* following exercise (Yang et al. 1996).

METHODS: C2c12 mouse myoblasts were seeded into 3D collagen lattices (4 million cells/ml: 75x25 mm) and cultured tethered (static endogenous tension) for 1 day in medium containing 10% Foetal Calf Serum (FCS), this was reduced to 2% FCS from day 2 to day 6, over which period myoblast fusion occurred, to give multinucleated myotubes. At the end of this period 3D constructs were attached in their long axis to the t-CFM for loading. Regimes used (i) cyclic loading (1% strain: 1-10 cycles/hour) (ii) ramp loading (10% strain: 1-10% per hour)

RESULTS: The force generation profile over 24 hours for myotube rich constructs was characterised by a series of rapid contractions(fig.1).

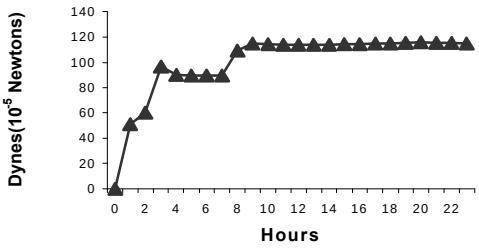

Figure 1. A typical myotube contraction profile. The total force produced, in this case was 115 dynes. Jumps are evident at 0 hours, 2 hours and 7 hours.

Various loading regimes were applied to constructs. It was found that IGF-1 was upregulated in constructs loaded with 10% strain ramp load (fig.3). Interestingly cyclical loading of 1% at 10 cycles per hour (fig.2), lead to a down-regulation in IGF-1.

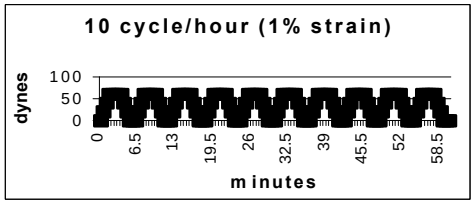

Figure 2. An example of a 10 cycle/hour loading regime applied to a myotube gel

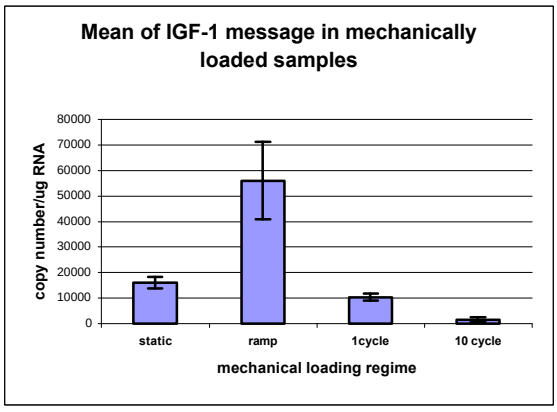

Figure 3. Changes observed in IGF-1 mRNA expression after various loading regimes were applied to skeletal myotube constructs.

DISCUSSION AND CONCLUSIONS: IGF-1 has been shown to be highly mechano-responsive in this simple model muscle. This response is discriminative between types of load, and distinct from responses by MGF (data not shown). These findings confirm the value of this model of skeletal muscle and suggest that it may have utility as the basis for engineered muscle constructs. It also supports the idea that cyto-mechanical cues may be

ideal for controlling tissue development by regulation of growth factor expression.

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REFERENCES: Florini, J.R., Ewton, D.Z. and Coolican, S.A.(1996) 'Growth hormone and the insulin-like growth factors system in myogenesis'. *Endo. Rev.* 17(5): 481-517. Prajapati, R.T., Eastwood, M., Brown, R.A. (2000) 'Duration and orientation of mechanical loads determine fibroblast cyto-mechanical activation: Monitored by protease release'. *Wound Rep. Reg*. 8:239-247. Yang, S., Alnaqeeb, M., Simpson, H. and Goldspink, G. (1996) 'Cloning and characterisation of an IGF-1 isoform expressed in skeletal muscle subjected to stretch'. *J.Muscle Res. Cell. Motility* 17: 487-495.