Use of an *in vitro* muscle model to investigate cellular and molecular aspects of exercise physiology: answering the key questions

D.J. Player[1], N.R.W. Martin[1], P. Davies[1], N. Sculthorpe[1], P.C. Castle[1], S. Passey[1], V. Mudera[3], R. Ferguson[4] & M.P. Lewis[1,2]

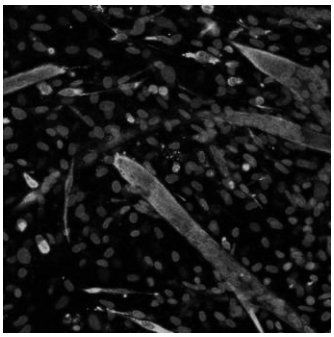
[1]Muscle Cellular and Molecular Physiology Group, Institute of Sport and Physical Activity Research, University of Bedfordshire, Bedford, [2]UCL Eastman Dental Institute, London, [3]UCL Institute of Orthopaedics and Musculoskeletal Science, and [4]School of Sport, Exercise and Health Sciences, Loughborough University .

INTRODUCTION: Research within exercise physiology has traditionally focused upon measurements of gross physiological function of skeletal muscle. However, in order to develop a greater understanding of the exact mechanisms that contribute to skeletal muscle in response to exercise, the cellular and molecular determinants need to be investigated. There is a growing body of *in vivo* research utilising methods of molecular biology, which has led to the establishment of proposed genes and proteins involved in the adaptation of skeletal muscle to exercise stimuli. *In vivo* exercise testing poses problems with regards to experimental control; accounting for inter-individual differences and methods relating to tissue sampling are common flaws of such research. *In vitro* models of skeletal muscle for investigating adaptation to exercise are in their infancy and generally lack biomimicity. It is therefore necessary to develop a model which has greater physiological relevance with respect to exercise, which encompasses the nature of the investigations currently underway in our laboratory.

METHODS: An established protocol was used for this experiment (Brady et al, 2008). Briefly, muscle derived cells (MDCs) were seeded in neutralised type-1 rat tail collagen and plated into a custom made multi-array 3D well system, containing five chambers. Each chamber held a custom built floatation bar ("A-frame") at either end. The collagen was allowed to gel in a standard incubator (37°C, 5% CO_2). Once set the collagen construct was cut away from the sides of the chamber and suspended in growth medium (20% foetal calf serum in high glucose DMEM). The "A-frames" provided two attachment points within the culture so that, as the cells attached and contracted, lines of longitudinal principle isometric strain developed. This tension provided sufficient mechanical stimulus to promote the realignment of the MDCs in a single plane. The result was a 3D tissue possessing uniaxially aligned and differentiated myotubes capable of performing

directed contraction. These models can be used for testing in their own right, or can be tethered to a culture force monitor, which allows real time analysis of force generation within the construct. An attachment of a stepper motor can also be made to the CFM (tensioning culture force monitor; t-CFM) to allow programmable regimes of mechanical strain to be applied to the construct. Once experimentation is complete the gels can be used for staining, protein analysis or PCR.

Fig. 1: 25 μm section through the 3D collagen construct, stained for desmin (green) and nuclei (blue).



STUDY DESIGN: This methodology is currently being used to investigate the following questions; (i). is an individuals' ability to respond to a resistance exercise stimulus predetermined by the number of muscle precursor cells one possesses? (ii). Do distinct signalling pathways exhibit a degree of 'cross talk' during adaptation to specific forms of exercise? (iii). Does the extracellular matrix impede the regenerative potential of aging skeletal muscle?

RESULTS: Immunohistochemical analysis confirmed the formation of primary myotubes (Fig 1). Preliminary PCR and CFM data suggests that the myotubes present in these 3D cultures is representative of *in vivo* muscle development and regeneration.

DISCUSSION & CONCLUSIONS: The components of the *in vitro* muscle model are in place to investigate cellular and molecular questions which remain to be elucidated in the field of exercise physiology.

REFERENCES: Brady et al., (2008), Synergy between myogenic and non-myogenic cells in a 3D tissue-engineered craniofacial skeletal muscle construct. *J Tiss Eng Regen Med*, 2(7) 408-417.