

Analysis of myotube-motoneuron interaction within an *in vitro* 3D collagen-based model of skeletal muscle.

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INTRODUCTION: In seeking to further our understanding of skeletal muscle physiology and function in both healthy and diseased tissues, there is a strong need to develop *in vitro* culture systems that better represent the *in vivo* condition. This project is aimed at developing an innervated 3D *in vitro* model of skeletal muscle. It is hoped that the incorporation of primary motoneurons into a 3D model of skeletal muscle established in our lab will promote myofibre development towards an adult phenotype and improve the biomimicry of the system. Furthermore, the formation of neuromuscular junctions (NMJ) within a 3D *in vitro* setting should allow for testing of the effects of neuromuscular agents in culture, thereby reducing the need for *in vivo* experimentation. Here we present data characterising the development and maturation of this 3D co-culture system in comparison to conventional 2D cultures and discuss the implications for the future of skeletal muscle tissue culture techniques.

METHODS: Muscle derived cells (MDCs) isolated from P1 neonatal rat pups were seeded in neutralised type-1 rat tail collagen and plated into standard dimension chamber slides (TTP Lab Tech). The slides each held a custom built floatation bar (termed “A-frame”) at either end. Once the collagen gelled, it was cut away from the sides of the chamber and suspended in growth medium (20% fetal calf serum in high glucose DMEM). This provided two attachment points within the culture so that, as the cells attached and contracted, lines of isometric tension developed along the long axis of the construct. 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 were cultured for 7 days before plating primary E14 rat motoneurons on top. The co-cultures were maintained for a further 7 days before being either a) immunostained for myogenic (eg desmin)

neuronal (eg MAP-2) and/ or NMJ (eg alpha-bungarotoxin, synaptophysin) markers or b) prepared for PCR analysis.

RESULTS: Immunohistochemical stains confirmed the alignment of MDCs in culture and their capacity to differentiate into primary myotubes. The capability of neurons to survive in the system was also verified. Ability for exploratory neurites to penetrate the collagen matrix and run parallel to the cultured MDCs was also observed (Fig. 1).

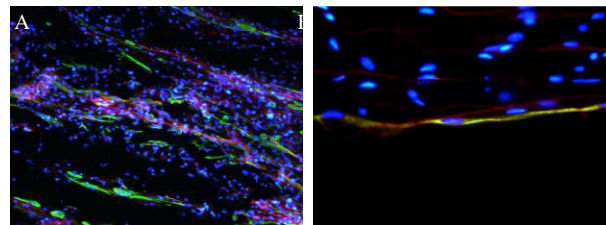


Fig. 1: 25 μm sections through the 3D collagen construct, stained for desmin (green) and MAP-2 (red, a neuronal marker) with a DAPI counterstain. Images taken at x20 (A) and x64 (B).

No significant difference was found by PCR in myogenin expression between 2D and 3D cultures, indicating that the fusion capability of MDCs in 3D is at least equal to that in standard cultures. The expression of synaptic markers synaptophysin, agrin and acetylcholine receptor suggests cells within this system are capable of forming synaptic contacts.

DISCUSSION & CONCLUSIONS: Though the formation of functional NMJs in this 3D model has yet to be established, our preliminary data indicates the presence and co-localisation of both pre and postsynaptic elements of NMJs. The survival of cells isolated from the ventral horn of E14 rat embryos within this system has been verified.

ACKNOWLEDGEMENTS: A.S.T. Smith is in receipt of an MRC studentship.