

## Establishment of a 3D engineered skeletal muscle-motoneuron co-culture using fibrin-cast gels

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**INTRODUCTION:** The successful *in vitro* engineering of complex tissues such as skeletal muscle would be of great benefit in the fields of tissue engineering and regenerative medicine, muscle physiology and neuromuscular research. A number of *in vitro* engineered models of skeletal muscle have been described that demonstrate many structural, biochemical and physiological similarities to *in vivo* muscle. As yet, however, there is no truly biomimetic *in vitro* model of skeletal muscle. An important aspect of *in vivo* muscle development and maintenance is the presence of a neuronal input via neuromuscular junctions (NMJs). Here we have increased the complexity of existing muscle models by introducing primary motoneurons with the aim of engineering a functional neuronal input in an established *in vitro* skeletal muscle model.

**METHODS:** The fibrin gel model of skeletal muscle culture has been described elsewhere (1) and fibrin gels were supplemented with 8µl/ml aprotinin. Rat MDCs were isolated from the hind limbs of P1 Sprague-Dawley rat pups by collagenase digestion, and cultured in fibrin gels until confluent (approximately 4-5 days) before the addition of MNs. MNs were isolated from the ventral horn of spinal cords from Sprague-Dawley rat E14 embryos and plated at 50,000 cells per gel. Co-cultures were maintained for up to 7 days prior to processing for immunocytochemistry or Q-PCR.

**RESULTS:** Microscopic analysis of skeletal muscle fibrin constructs revealed the formation of tight bundles of myotubes aligned along the long axis of the construct and often displaying the striated pattern characteristic of skeletal muscle. Expression of genes involved in muscle cell differentiation (myogenin) and contraction (Myosin heavy chain, MYH3) was higher in 3D fibrin constructs compared to conventional 2D culture, illustrating the improved maturation of the

muscle constructs in 3D fibrin cast gels. MNs survive within the co-culture system neurites could be seen extending through the construct in association with myotubes (Figure 1). Preliminary data indicates that co-culture of MDCs with primary MNs results in increased expression of the NMJ genes acetylcholine receptor (AChR) and Choline acetyltransferase (ChAT).

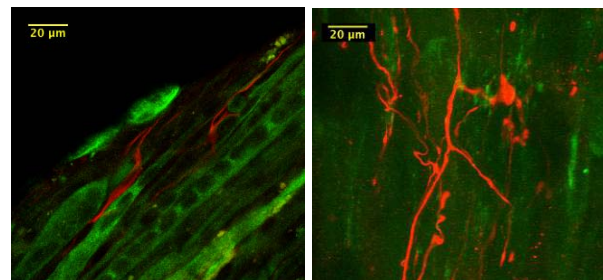


Figure 1. (A) MN neurites (red) can be seen extending in association with the myotubes (green) within fibrin-cast skeletal muscle constructs. (B) Maximum intensity projection from a confocal z-stack showing a network of neurites extending in 3 dimensions within the muscle construct. Scale bar = 20µm.

**DISCUSSION & CONCLUSIONS:** Using an established model of skeletal muscle, we have added extra complexity through the addition of a neuronal input. Fibrin cast skeletal muscles can be rapidly engineered together with MN, producing contractile neuromuscular constructs. The presence of MNs in the co-culture system induces the expression of NMJ genes within the muscle cells indicating physiological interactions between the two cell types which represent the first step towards the formation of organized NMJs. Establishment of a 3D muscle-MN culture system will be of great benefit to the study of NMJ formation, maturation, and function and will reduce reliance on animal models for such studies.

**REFERENCES:** <sup>1</sup> Rapid Formation of functional muscle *in vitro* using fibrin gels. Yen-Chih Huang, et al. J Appl. Physiol. 98:706-713, 2005.