Mechanical Behaviour of Primary Human Skeletal Muscle Cells and Isolated Non-Myogenic Cells Within a 3D- Construct

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INTRODUCTION: An understanding of the mechanical and mechano-molecular responses that occur during the differentiation of primary human myoblasts in 3D-culture is critical for understanding growth and progress towards producing a tissue-engineered muscle construct[1]. In an effort to characterise the mechanical behaviour of myoblasts within a 3D construct, previous work by Cheema et al. (2003) utilised a cell line of immortalised skeletal myoblasts (c2c12 cells). The aim of this study was to determine the mechanical response of primary human skeletal muscle (PHSM) cells. Further, to isolate myoblasts from PHSM cells and establish the force generated by those cells.

METHODS:

Masseter muscle biopsies were obtained from consented healthy patients undergoing orthognathic surgery at the Eastman Dental institute. The expanded PHSM cells were subsequently defined as a co-culture of CD56+ve (adult human myoblasts) and CD56-ve cells. Primary human myoblasts were isolated using microbead-immunomagnetic selection (CD56+ve). The efficacy of this technique was verified by immunostaining. The co culture of primary human muscle cells, isolated CD56+ve cells and CD56-ve cells respectively were seeded in collagen constructs (1million/ml) and the force generated by the cells was quantified over 24 hours.

RESULTS:

The mechanical response of PHSM cells in a 3D construct reveals an average peak force generation of 50 dynes (*Fig.1a.*). PHSM myoblasts (CD56+ve) and non myogenic (CD56-ve cells) were successfully isolated (*Fig. 2.*) and the non-myogenic cells also generated an average peak force of 50 dynes over 24 hours (*Fig. 1b.*).

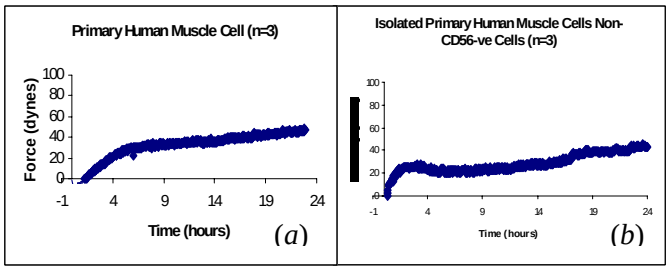

Fig. 1: Contraction profiles of (a) primary human skeletal muscle cells and (b) isolated non-myogenic cells.

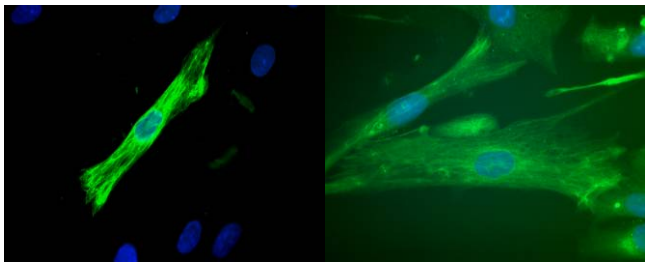

Fig. 2: Immunostaining confirming the isolation of human myoblasts (CD56+ cells) from primary human skeletal muscle (green immunostaining).

DISCUSSION & CONCLUSIONS:

We have shown that there are no differences in force generation and contraction of 3D collagen constructs by PHSM's and CD56-ve cells. The mechanical characterisation of isolated primary human CD56+ cells and future differentiation of these cells into myotubes within a 3D construct will further understanding of muscle growth and regeneration and aid in defining parameters for functional muscle engineering.

REFERENCES:

[1]U. Cheema, S-Y.Yang, V.Mudera, G.G. Goldspink, R.A. Broan (2003) 3-D In Vitro Model of Early Skeletal Muscle Development. *Cell motility and the cytoskeleton* **54**:226–236

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