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Small-scale skeletal muscle constructs for in-vitro musculoskeletal junction preclinical testbed [Abstract]

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Small-scale skeletal muscle constructs for *in-vitro* musculoskeletal junction pre-clinical testbed.

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INTRODUCTION: Currently, musculoskeletal tissue damage is surgically repaired with the aim of restoring functionality over form. Any resulting tissue damage reduces utility and can increase the likelihood of further local injury. Therefore, more effective repair and regeneration techniques are needed.

By creating an *in vitro* musculoskeletal junction that closely replicates the form and function of the *in vivo* system, novel materials and chemical products could be tested for viability in a more representative manner.

In order to engineer a repeatable system capable of scaling-up, a small scale model of bone and muscle must be optimised.

METHODS: 0.75mL 3D type-1 rat-tail collagen (2.20mg/mL) neutralised constructs were seeded with C2C12 murine myoblast cells at 4×10^6 cells/mL as previous described (Sharples *et al.* 2012) and set in a gelling area of 11mm x 14mm at 37°C, 5% CO₂. The constructs were tethered at either end by bespoke polythene mesh floatation bars to create longitudinal lines of isometric tension (*Fig 1*). Constructs were placed in 20% FBS high glucose DMEM for 4 days and then cultured in 2% horse serum high glucose DMEM to induce differentiation.

RESULTS & DISCUSSION:

Immunohistochemical staining for the intermediate filament protein Desmin showed the capacity for alignment and differentiation of C2C12 myoblasts within the collagen system at this scale (*Fig 1*). This replicates at a molecular level what is seen in larger constructs (Sharples *et al.* 2012).

CONCLUSIONS: Preliminary indications show that a small-scale tissue engineered muscle suitable for a preclinical test-bed is viable from a phenotypic standpoint. Myotubes are evident and aligned however genotypic considerations and co-cultured limitations with osteoblasts are yet to be taken into account.

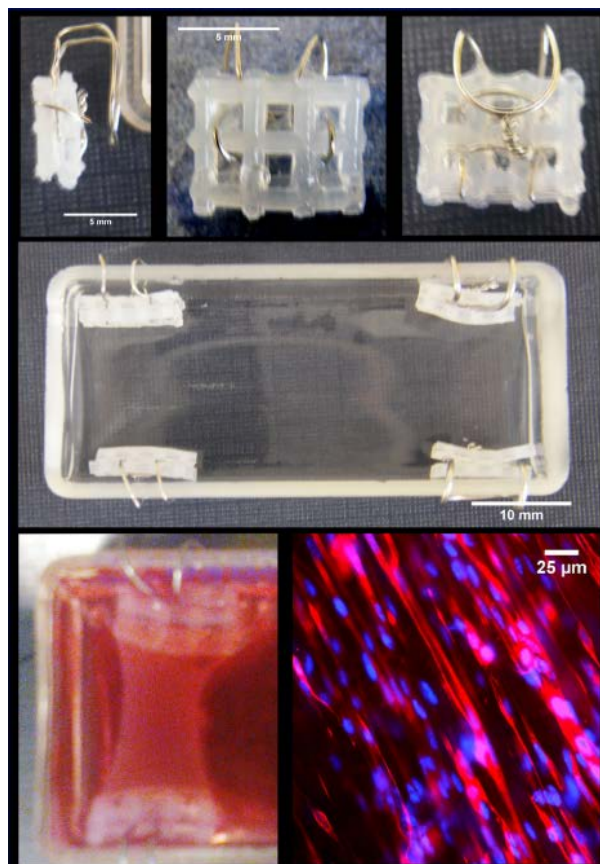


Fig. 1: Collagen Muscle System: 0.75ml collagen/cell hydrogel set between PTFE 'floatation bars'. Immunohistochemistry shows Desmin intermediate filament (red) and DAPI nuclear stain (blue) taken at edge of the gel (40x magnification).

REFERENCES: Sharples, A. P., Player, D. J., Martin, N. R. W., Mudera, V., Stewart, C. E., & Lewis, M. P. (2012). Modelling *in vivo* skeletal muscle ageing *in vitro* using three-dimensional bioengineered constructs. *Aging cell*, 11(6), 986–95.

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