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Preclinical musculoskeletal junction testbed: co-culture of 3D tissue engineered skeletal muscle and bone constructs

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INTRODUCTION: Current preclinical tests utilize a combination of monolayer cultures and animal models to assess biocompatibility[1]. This is despite ethical considerations, a lack of cross-species translation, cost and non-relevant structural and biochemical pathways. Therefore, a relevant tissue engineered (TE) model of the musculoskeletal system is proposed (MSk). In order to create a relevant 3D TE preclinical MSk testbed, each component (skeletal muscle and bone constructs) must first be capable of simultaneous culture in a single contiguous system. This work presents conditions for the successful co-culture of 3D tissue engineered skeletal muscle and bone models.

METHODS: C2C12 murine muscle precursor cells (MPC) and TE85 human osteosarcoma cells were seeded within type-1 rat-tail collagen (2.20mg/mL) and collagen/nano-hydroxyapatite constructs respectively. Nano-hydroxyapatite (nHA) was precipitated through the reaction of calcium acetate and ammonium phosphate tribasic solutions. After removal of unbound water, the nHA was mixed with the collagen prior to cell seeding. Skeletal muscle (C2C12) constructs (SkM) were tethered at either end by bespoke polythene mesh floatation bars to facilitate alignment of MPC's and subsequent differentiation into myotubes. Bone (TE85) constructs (B) were set within a bespoke circular mould and restrained by a centrally located pin and bead. Both constructs were placed in 20% FBS high glucose DMEM for 4 days and then cultured in 2% horse serum high glucose DMEM for a further 10 days; these being the standard conditions to induce MPC differentiation.

RESULTS: Both SkM and B constructs reduced in width and diameter over the 14 day time-course, indicating successful cell attachment and remodelling. Immunohistochemical analysis showed successful myotube formation within the skeletal muscle construct and a randomly orientated, osteocyte-like morphology with clear cell protuberances within the bone model.

DISCUSSION & CONCLUSIONS: This work demonstrates the successful simultaneous co-culture of tissue engineered skeletal muscle and bone within a single system. This data shows conservation of previously reported morphological observations of a collagen-based skeletal muscle construct [2, 3] without obvious negative effects from the adjacent bone culture. The bone construct similarly showed no deviation from expected observations and therefore shows potential for muscle and bone co-culture within a contiguous construct.

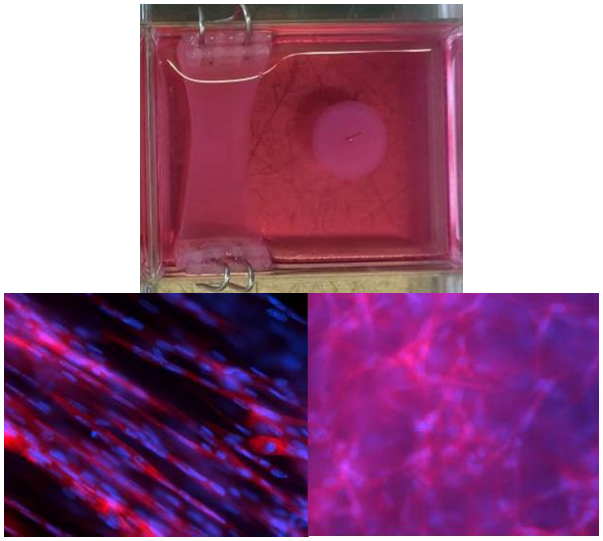

Fig. 1: (Top) Collagen based skeletal muscle and bone constructs cultured in a single well for 14 days. (Bottom Left) 40x mag. Parallel alignment of C2C12 myotube structures within skeletal muscle construct. (Bottom Right) 40x mag. Randomly aligned osteocyte-like morphology of TE85s within collagen scaffold. Immunohistochimistry: Phalloidin (actin) - Red; DAPI (nuclei) – Blue.

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