Preclinical Musculoskeletal Junction Testbed: Optimisation of a Reproducible Skeletal Muscle Construct

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INTRODUCTION: Prior to new material or drug use in humans, a series of preclinical and clinical tests to determine compatibility with human subjects must be passed1. Tissue engineered constructs allow for high throughput testing in a tightly controlled environment whilst maintaining strong mimicry of in vivo architecture. However, this is often costly in terms of resources and cell numbers. Therefore, it was sought to optimise the manufacturing variables to limit use of resources whilst maintaining characteristics of the in vivo tissue. This work proposes that variation in cell density (2, 3 and 4 million cells/mL) and alterations in attachment forces governed by gel width and surface area of attachment will regulate the formation and maturation of tissue engineered skeletal muscle constructs.

METHODS: 3D type-1 rat-tail collagen (2.20mg/mL) neutralised constructs were seeded with C2C12 murine myoblast cells as previously described3. 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. Preclinical work relies on high numbers of replicates; therefore, this study is conducted in a scaled down model (0.3mL-0.8mL) to reduce resource use whilst maintaining basic characteristics of the previous model3 (3mL total volume)

RESULTS: Variation between 2, 3 and 4 million cells/mL of collagen and construct designs (volume and attachment area variation) had no significant effect on contraction time or width reduction (ANOVA $P>0.05$). Preliminary analysis indicates that the width of the construct has an effect on alignment and higher cell densities produce greater alignment. Physical success of the muscle construct with an extension past the anchor point is 30% greater than without [~100% (n=6) vs. ~70% (n=6)]. High cell densities displayed greater number of unattached cells. Immunohistochemical staining for the intermediate filament protein Desmin showed the capacity for alignment and differentiation of C2C12 myoblasts within the collagen system (Fig. 2).

Fig. 1 Muscle construct variables: Observed effects of initial width past anchor point and cell density. (left) Day 0 (right) Day 14 (top), 4million cells/mL (0.8ml, ~15mm initial width) (bottom) 2million cells/mL (0.3mL ~5mm initial width)

Fig. 2. Immunohistochemistry shows Desmin intermediate filament (red) and DAPI nuclear stain (blue) (40x magnification).

DISCUSSION & CONCLUSIONS:

The optimum extension lies between 0%-50% of the anchor point width based on alignment and physical success of the construct. This data replicates published work in a similar model3 whilst expanding previous alignment data4. Further phenotypic and genotypic analysis is to be completed.

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