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Materials 3D Printed via fused deposition modelling elicit myofibrillar alignment and enhanced differentiation of skeletal muscle cells *in-vitro*

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INTRODUCTION: 3D printing allows the generation of physical devices within short time-periods from bespoke CAD designs. 3D printing technologies such as selective laser sintering (LS) and stereolithography (SL) are now being utilised within biomedical engineering for the fabrication of microfluidic devices[1] and scaffolds for living cells[2], using what is hoped are biodegradable and biocompatible 3D printed materials. If these technologies are going to form the platforms within which complex cellular physiological process are to be re-created then understanding and defining the compatibility between various mammalian cell types and the printed materials is of paramount importance. This investigation sought to examine the biocompatibility of C_2C_{12} muscle cells on commercially available polymers; poly-lactic acid (PLA), acrylonitrile butadiene styrene (ABS), polyethylene terephthalate (PET) and polycarbonate (PC) when 3D printed via FDM.

METHODS: Biocompatibility experiments were designed to simultaneously assess both the direct (cells cultured directly on the material) and in-direct (cells cultured in chemically leached medium) effects of each polymer on cellular viability, morphology and differentiation after 3 days in GM (GM72hrs) and 5 days in DM (DM120hrs). C_2C_{12} muscle precursor cells (MPCs) were seeded into eight six-well plates at a density of $6x10^5$ cells for each repeat (n=3). Each well/six-well plate was considered n=1 per condition (Control & Material), per analysis at each time-point totaling n=18 per analyses. This protocol was completed separately for each polymer (PLA, ABS, PET and PC). After alamarBlue® cellular viability analysis was conducted, cells were stained for the actin cytoskeleton and nucleic DNA in addition to being harvested for PCR assays.

RESULTS: Polymer dependent reductions in cell viability were observed when cultured directly on 3D printed materials (GM72hrs: PLA; $P\leq0.05$, PC; $P\geq0.05$, DM120hrs: PLA; $P\leq0.05$, PC; $P\leq0.05$). Cells cultured on PET also showed reduced viability ($P\leq0.05$); however this was not evident in cells cultured on ABS ($P\geq0.05$). In-direct observations outlined increases in cell viability when cultured in leached constituents of PLA after DM120 ($P\leq0.05$) and ABS after GM72

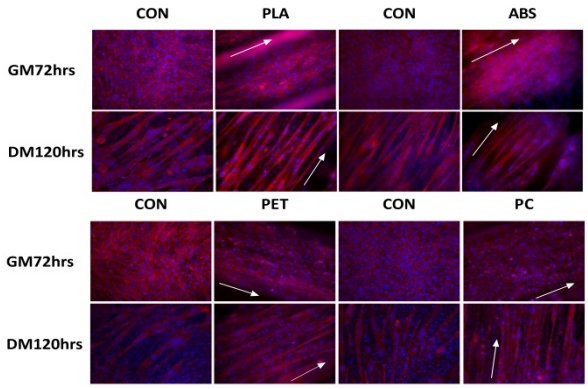

Fig.1: Morphological staining of the actin cytoskeleton (red) & nucleic DNA (blue) for PLA, ABS, PET, PC and respective controls at GM72 & DM120 time-points.

($P\leq0.05$), however this was not evident in PET ($P\geq0.05$) or PC ($P\geq0.05$).

Morphological observations (*Fig.1*) outlined reduced myotube widths in cells cultured on PLA ($P\leq0.0001$), PC ($P\leq0.05$) and PET ($P\leq0.0001$) however this was not apparent in ABS ($P\geq0.05$). Thinner myotubes were observed in cells cultured in chemical leachable of ABS ($P\leq0.05$), however no difference was observed between other in-direct conditions. C_2C_{12} cultured on 3D printed materials also exhibited significant nucleic (PLA; $P\leq0.0001$, ABS; $P=\leq0.05$, PET; $P=\leq0.05$, PC; $P\leq0.0001$) and myofibrillar alignment (PLA; $P\leq0.0001$, ABS; $P\leq0.05$, PET; $P\leq0.05$, PC; $P\leq0.0001$).

Augmented myogenin $\Delta\Delta C_T$ expression was documented at early time-points; GM72 in PLA ($P\leq0.0001$), ABS ($P\leq0.05$) and PC ($P\leq0.0001$), however this was not observed in PET ($P\geq0.05$). Myogenin remained potentiated after DM120 in PLA ($P=\leq0.05$), however this response was reduced in ABS ($P\geq0.05$), PET ($P\geq0.05$) and PC ($P\geq0.05$). No difference was observed in cells cultured in the leached medium of materials.

DISCUSSION & CONCLUSIONS: This investigation observed biocompatible polymers 3D printed via FDM which exhibit morphological alignment and enhanced differentiation of C_2C_{12} cells, providing rationale for use when re-creating platforms for cellular physiological processes.

REFERENCES: [1]Kitson, P.J. et al., 2012. *Lab on a Chip*, 12(18), p.3267.[2]Lantada, A.D. et al., 2012. *Plasma Processes and Polymers*, 9(1), pp.98–107.