SUSPENDED MANUFACTURE OF BIOLOGICAL STRUCTURES

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We present a novel method of extrusion-based ALM for the production of cell-laden strucutres from low viscosity polymers. The traditional planar print bed is replaced with a bed of micoparticulate fluid gel. During the extrusion process, the fluid gel is displaced whilst providing a support strucutre for the low viscosity material allowing manufacture of relatively complex geometries. The extruded structure can then be easily removed from this self-healing fluid bed. For this study, a bi-layered cell-seeded construct was produced to model the osteochondral junction.

Osteochondral plugs were produced by the addition of chondrocytes and osteoblasts to 1.5%w/v gellan and 1.5%w/v gellan-5% nano-hydroxyapatite respectively. The consecutive extrusion of these two solutions into the fluid bed followed by further ionic crosslinking produced the bi-layered construct that was implant into a femoral condyle defect *in vitro*.

Cell viability following extrusion was confirmed using calcein AM/PI live/dead staining showing excellent viability. Constructs were then sectioned, and qRT-PCR was performed, showing a native collagen phenotype across the construct with evidence of matrix markers in the cartilage-like region which were also identified using fluroescent-IHC.

Constructs were also tested for their bulk relaxation properties. Addition of nano-hydroxyapatite in the bone-like region resulted in a faster, more elastic relaxation than gellan alone, something that has previously been reported to favour osteogenic differentiation.

We have demonstrated the efficacy of suspended manufacturing to maintain viability and phenotype of two populations of human primary cells in a single construct thus emulating the structure of the osteochondral junction.

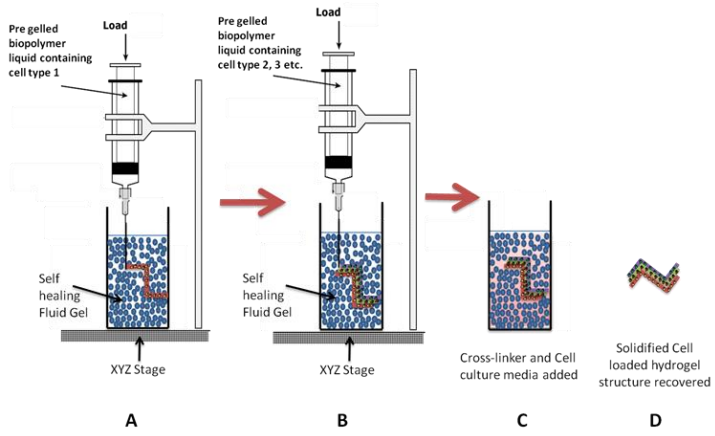

Figure 1 – Suspended manufacturing process

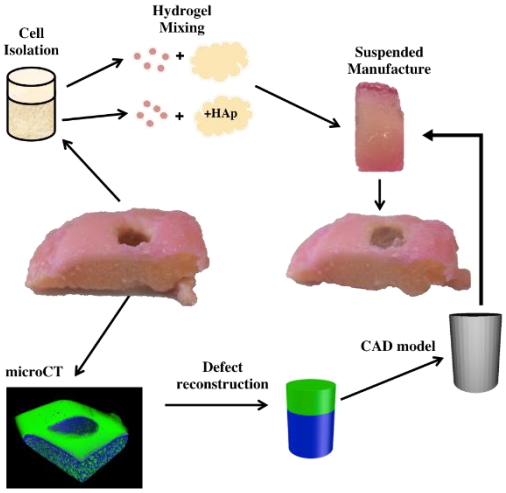

Figure 2 – Suspended manufacturing workflow for an osteochondral plug