Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Title: Compartmentalized Bioencapsulated Liquefied 3D Macro-Construct by Perfusion-Based Layer-by-Layer Technique.

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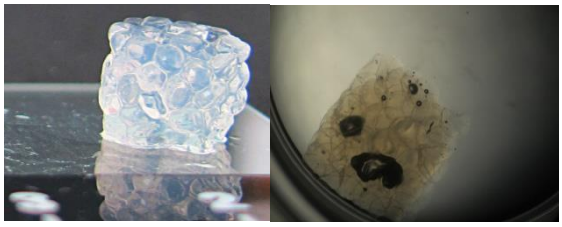
3D macro-constructs were produced by assembling individual Ca-Alg microspheres, obtained from ionotropic gelation, acting as building blocks, by using a novel perfusion-based layer-by-layer technique. This two-step process starts with producing microspheres that are grouped together to form a 3D core structure followed by liquefaction to obtain liquefied 3D macro-structures. Below are the group images of the 3D constructs with different external geometry by the optical microscope during the progression of liquefaction process– **A-D**. The visual change in the density of beads confirms liquefaction process. All constructs display good integrity.

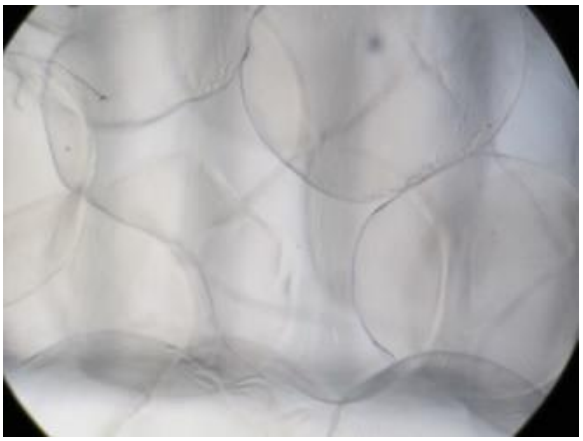
A

B

C

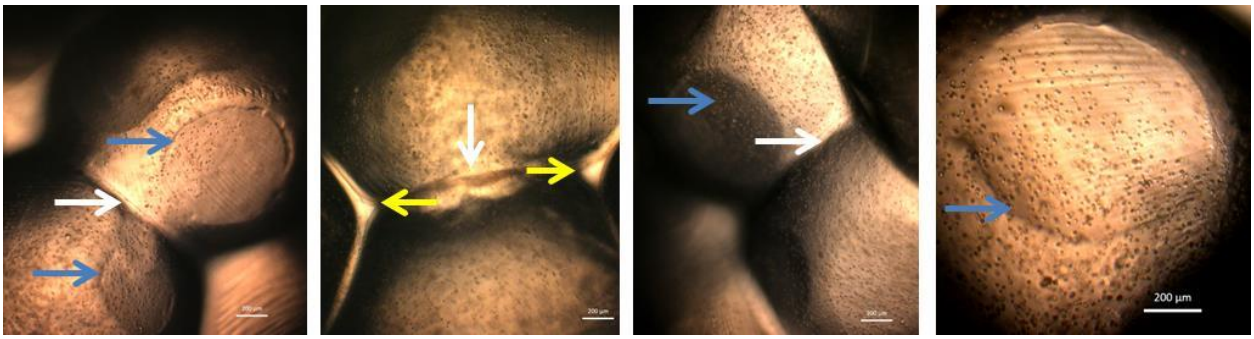


D



At higher magnification coated multilayer follow the exact negative replica of the beads with defined connections at the point of contacts of microspheres in 3D core structure.

Imaging of these macro-constructs was difficult and thereby restricted to optical and florescence microscopy because of the dimensions and hydrogel nature of construct. Different tactic was employed by using transmitted light after performing live-dead assay. The better display of surface characteristics was obtained from the microspheres that were decisively detached. The interpretation of these pictures clearly supported our hypothesis about maintaining of inter-gaps as well as defined multilayer bridges at the junctions of hydrogel microspheres. The severed microspheres displayed clear impressions of the broken multilayer connections which bind them together. This interesting observation may lead us to presume about increasing intranetworking between the individual microspheres which can be restricted for those with intact core. This feature may well be very helpful for the purpose of TE particularly in tissue formation ex-vivo. Apart from this it also suggests the stability of the construct during the process of experiment.



E F G H

E- G: Optical pictures taken of the 3D constructs of 7th day after performing live dead assay. Colored arrows refer to, White-Multilayer bridges, Yellow - gaps and Blue – Imprints of broken multilayer connections. H: Single hydrogel bead from liquefied construct showing the area of connectivity. (Scale bars represent 200 µm).