Supplementary Information for:

High-throughput fabrication of hepatic cell clusteroids with enhanced growth and functionality for tissue engineering applications

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Content

Droplet shrinking effect of adding PEO to the W/W Pickering emulsion

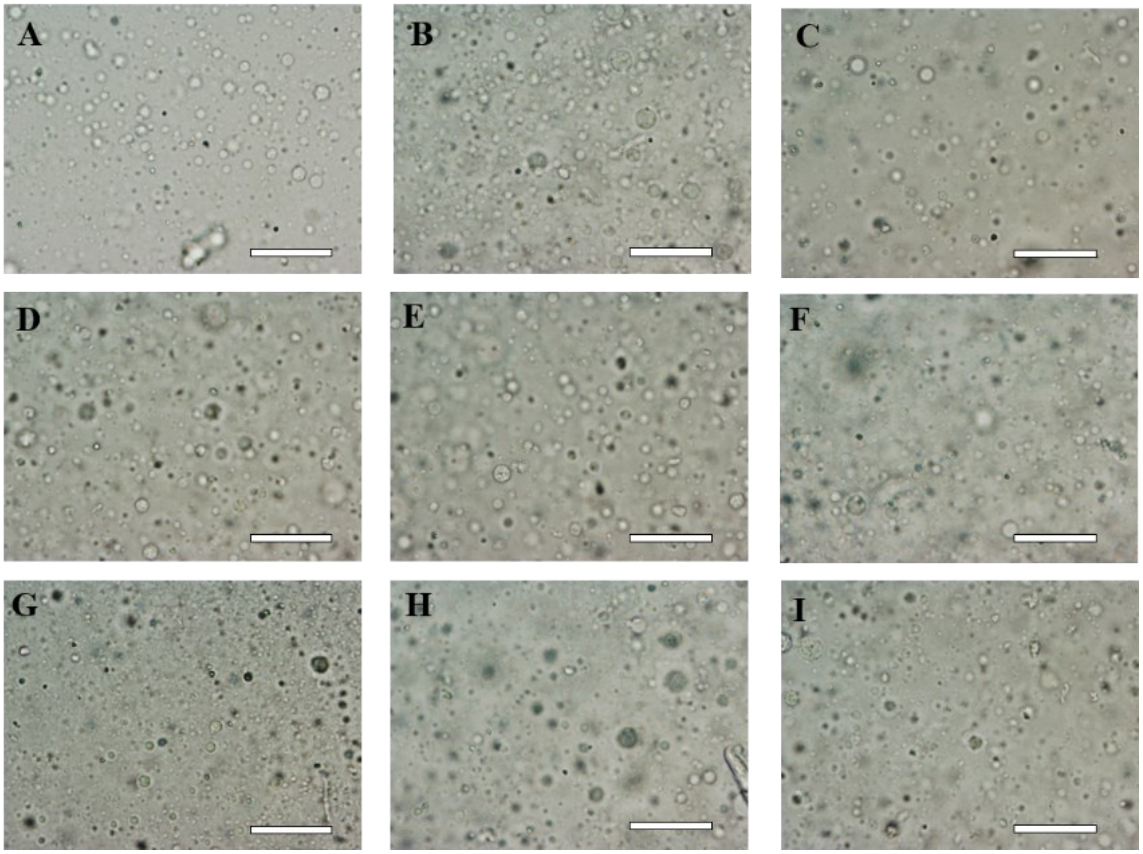

Figure S1. Optical microscopy images of a DEX/PEO water-in-water Pickering emulsion (PEO 5.5wt% and DEX 5.5 wt%) after shrinkage. (A) ϕ Dex = 0.5, (B) ϕ Dex = 0.33, (c) ϕ DEX = 0.25, (c) ϕ DEX = 0.2 (c), ϕ DEX = 0.17 (c), ϕ DEX = 0.14, ϕ DEX = 0.125, ϕ DEX = 0.11 and ϕ DEX = 0.1, stabilized by 2wt% WP particles at pH 6.18. Scale bars are 100 μm.

Schematics of the formulation of Hep-G2 clusteroids

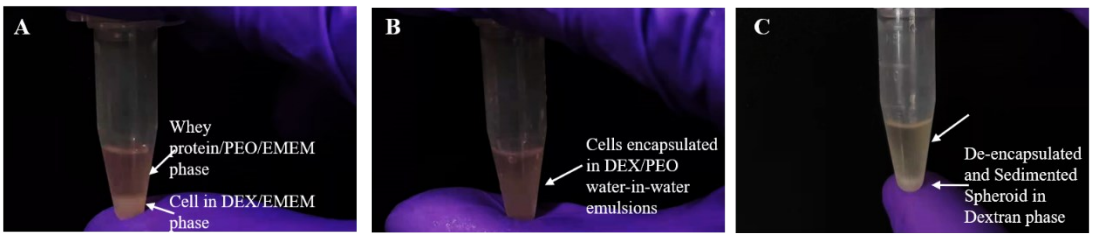

Figure S2. Schematics of the formulation of Hep-G2 clusteroids in water-in-water Pickering emulsions.

Clusteroids' viability at different days of culture

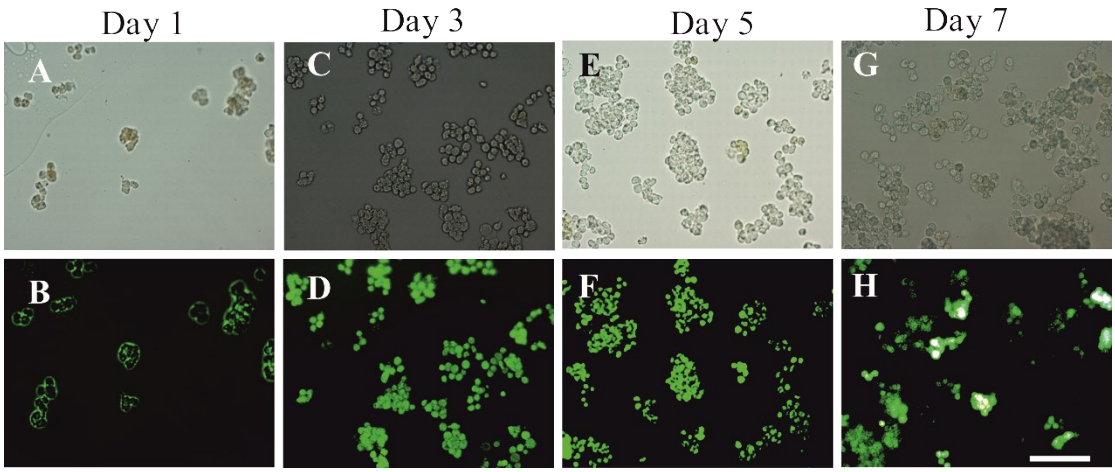

Figure S3.(A,C,E,G) Optical bright-field images and (B,D,F,H) fluorescence microscope images of Hep-G2 clusteroids after being treated with FDA live/dead assay after various days of culture. The fluorescence indicates that both the Hep-G2 cells preserve their viability during the clusteroids fabrication process. The scale bar in (H) is 100 μm and is the same for all images (A-F).

Long term culture of clusteroids in the hydrogel matrix

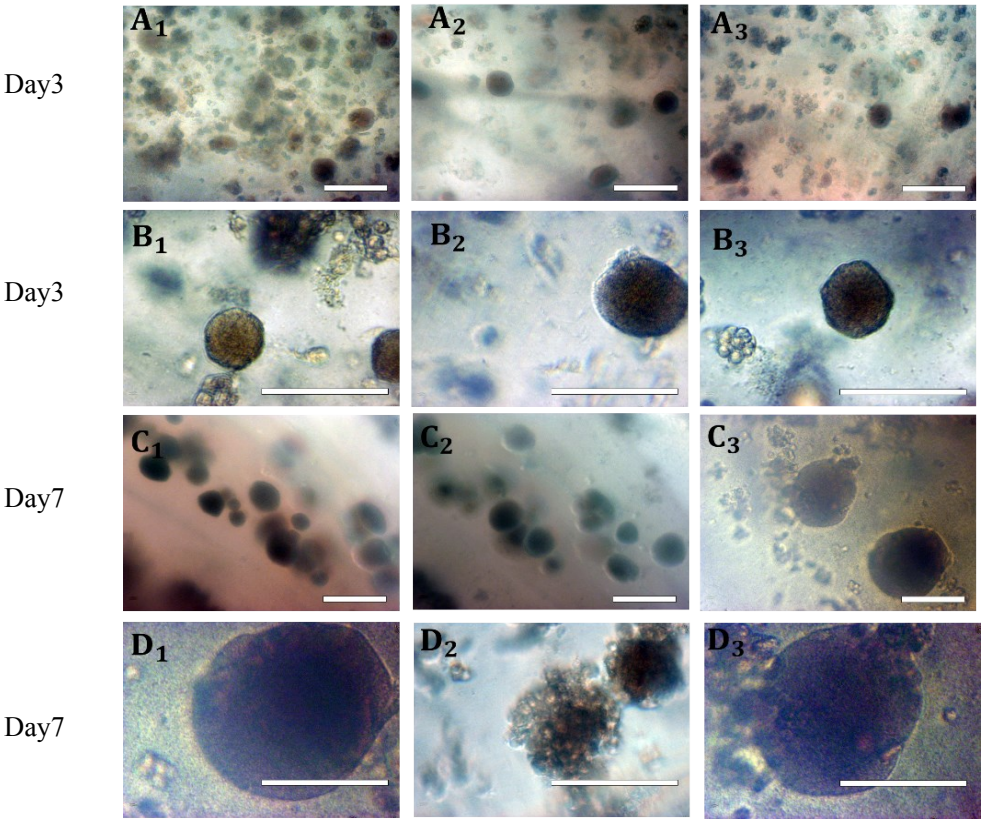

Fig. S4. Bright-field optical microscope images of (A, B, C, D) 3D cultured Hep-G2 clusteroids isolated by a dilution of the DEX/PEO emulsion by a factor 3 with EMEM medium and incorporated with 1 wt% sodium alginate in EMEM media followed by cross-linking with 1M $CaCl_2$. The Hep-G2 cells clusteroids were cultured in the alginate film for seven days under EMEM media and images were taken from each well to determine the average clusteroids size. Scale bars are 100 µm

Urea and albumin production of clusteroids at different days of culture

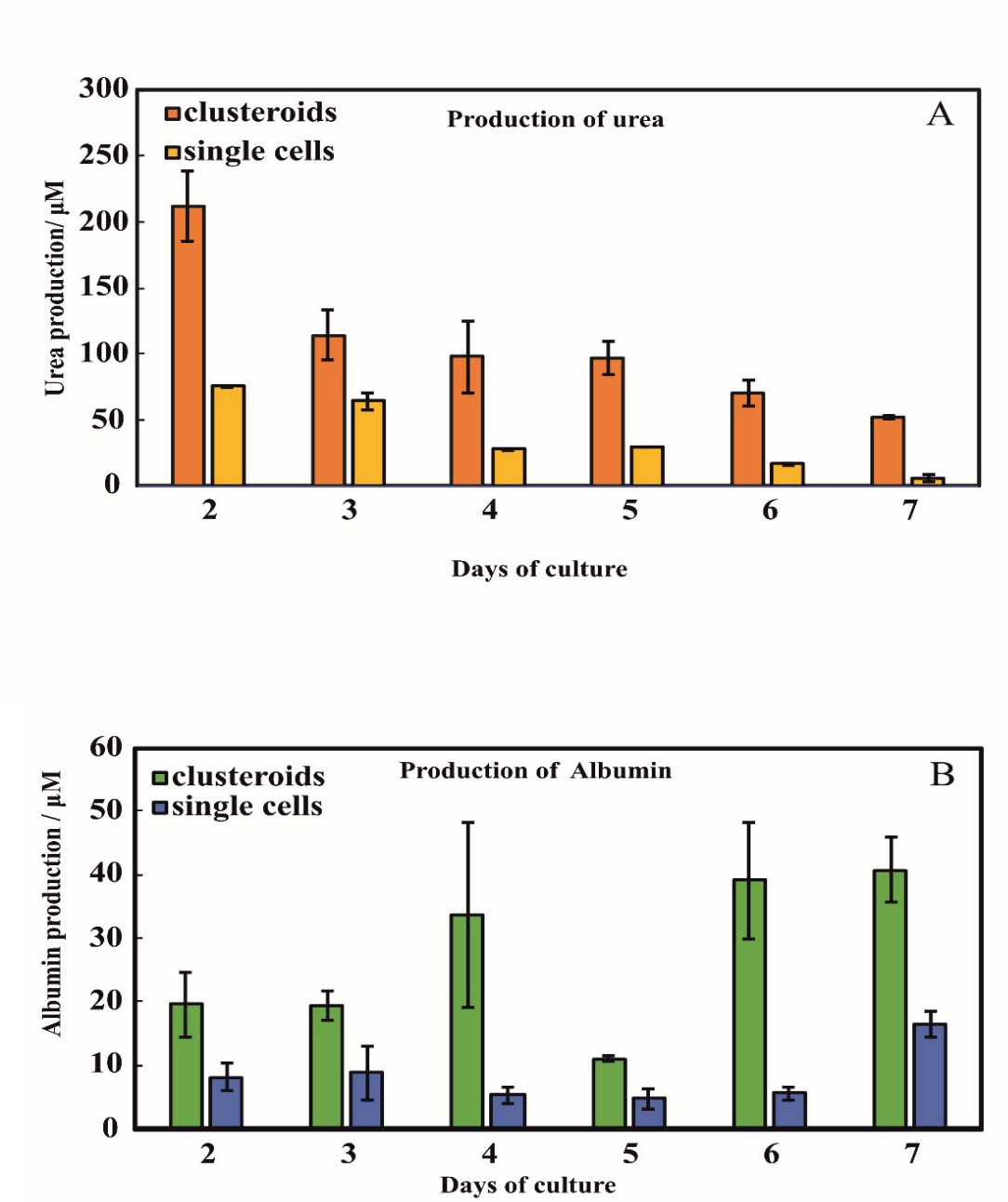

Fig. S5. (A) Albumin secretion and (B) urea synthesis by Hep-G2 cells in blend gels as a function of culture time by days. Data are shown as mean ± standard deviation from two samples. The superscript letters represent significant difference between groups.