Supporting Information

Planar and Cell Aggregate-Like Assemblies Consisting of

Microreactors and HepG2 Cells

Yan Zhang,[#] Philipp S. Schattling, [#] Fabian Itel, [#] Brigitte Städler^{*,#}

Dr. Yan Zhang, Dr. Philipp S. Schattling, Dr. Fabian Itel, Dr. Brigitte Städler Interdisciplinary Nanoscience (iNANO), Aarhus University, Gustav Wieds Vej 14, 8000, Denmark E-mail: bstadler@inano.au.dk



Figure S1. Bright field images of live co-cultured HepG2 cells and Alg particles at different number ratios (cell to Alg particle = 25/1 and 1/1) for 24 h, 48 h and 72 h. For better visualization, the particles are highlighted in selected areas of the images. The scale bars are 200 µm.



Figure S2. Bright field images of fixed co-cultured HepG2 cells and Alg particles at different number ratios (cell to Alg particle = 25/1 and 1/1) for 24 h, 48 h and 72 h. For better visualization, the particles are highlighted in selected areas of the images. The scale bars are 100 μ m.



Figure S3. Representative CLSM images, taken at different focal planes, of uncoated Alg particles cocultured with HepG2 cells for 72 h (cell to Alg particle = 25/1). The scale bars are 20 µm.



Figure S4. Surface coating: Bright field images of fixed co-cultures between HepG2 cells and and Alg^+ (a) and Alg^c (b) particles (cell to particle ratio 25/1) with different surface coatings after 24 h, 48 h and 72 h. The Alg particles are highlighted for visualization purposes. The scale bars are 200 μ m.



Figure S5. Number of particles per mm^2 for different cell to particle ratios using (uncoated) Alg, Alg⁺ and Alg^c after 24 h, 48 h and 72 h incubation time with HepG2 cells.



Figure S6. Bright field images and sizes of cell aggregates assembled from HepG2 cells and ~65 μ m Algc particles in a cell to particle ratio of 25/1 (i) or 10/1 (ii) after 24 h incubation time. The scale bars are 500 μ m.



Figure S7. Representative CLSM images of cell aggregates only (a), aggregates containing cell and Alg^c in a ratio 25/1 (b) and 10/1 (c) after 3 d (top row) and 7 d (bottom row). The images in a sequence were taken at the same x-y location in different z-planes (separation between the z-planes left to right was ~70 μ m). (Blue: DAPI stained nuclei; green: PLL_F of the coated Alg^c, red: phalloidin stain). The scale bars are 20 μ m.

a) Cells only



Figure S8. Representative CLSM images of live (green) – dead (red) stained cell aggregates assembled from HepG2 cells only (a) or HepG2 cells and Alg^c in a cell to particle ratio of 25/1 (b) or 10/1 (c). Images were taken after 7 d at the same x-y location in different z-planes (separation between the z-planes left to right was ~70 μ m). The scale bars are 20 μ m.



Figure S9. a) The normalized fluorescence intensity of a H₂O₂-containing solution assessed by the Amplex red assay after being exposed to AlgLcat and Algcat for 30 min at 37 °C. (40 μ M H₂O₂ starting concentration, 25000 microreactors per mL used, t₀: microreactor directly after assembly, t₂₄: microreactors after 24 h HEPES1 buffer incubation at 37°C, n = 3) b) The normalized fluorescence intensity of a H₂O₂-containing solution assessed by the Amplex red assay (at room temperature RT) after being exposed to AlgLcat and Algcat for 30 min at room temperature or 37 °C. (40 μ M H₂O₂ starting concentration, 10000 microreactors per mL used, n = 3)



Figure S10. Dose response curves of HepG2 cells exposed to different concentrations of H_2O_2 for 24 h.