Supplementary information

Microcapsules with a permeable hydrogel shell and an aqueous core continuously produced in a 3D microdevice by all-aqueous microfluidics

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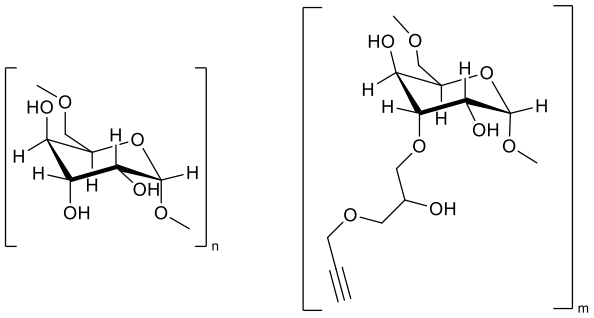
Synthesis of modified dextrans

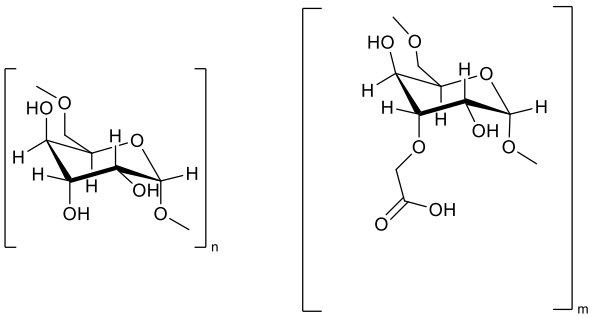
Dextran-GPE



Scheme S1. Structure of Dextran-GPE.

The glycidyl propargyl ether derivatized dextran (DEX-GPE, MW = 500 kDa, Scheme S1) was prepared using the procedure of Nielsen *et al*.[1] The degree of substitution of DEX-GPE was determined by ^{1}H NMR as 0.075 (Fig. S1).
^{1}H NMR (400 MHz, D_2O), δ ppm: 5.34, 5.19, 5.00 (anomeric CH, 1H); 4.28 (O-CH_2-C, 2H, s); 4.15-3.45 (6H, m).
^{13}C NMR (100 MHz, D_2O), δ ppm: 100.56 (C1), 76.25 (C3), 74.26 (C2), 73.57 (C5), 73.04 (C4), 68.39 (C6), 61.15.

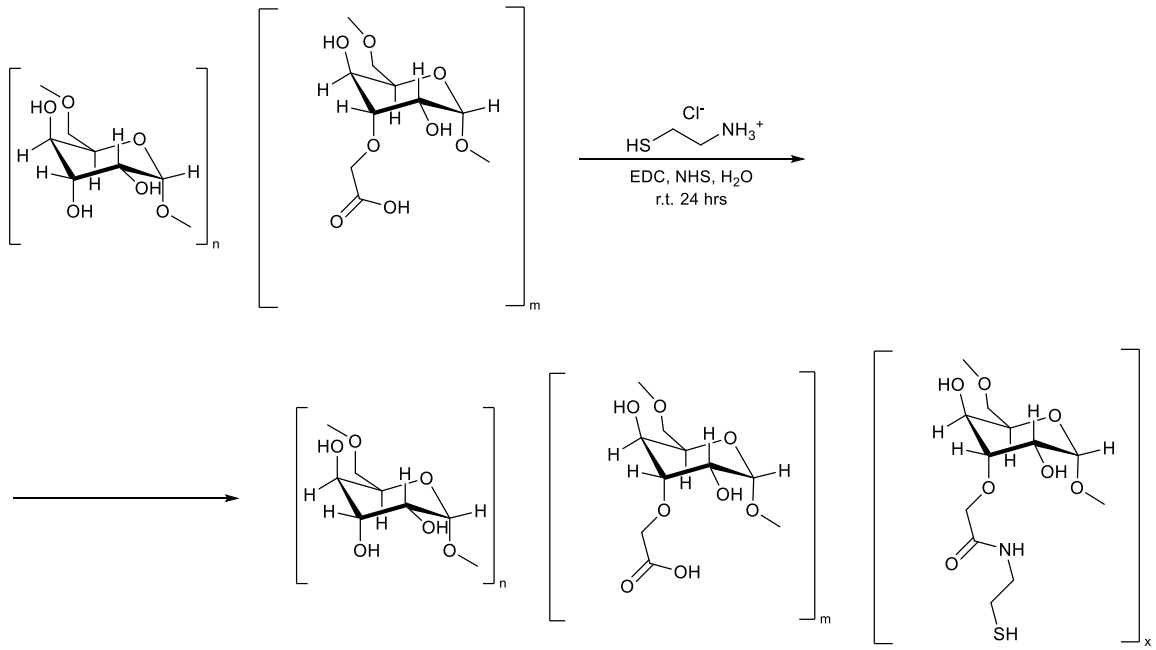
CM-dextran (CM-DEX)



Scheme S2. Structure of CM-dextran.

Carboxymethylation of dextran (MW = 20 kDa, scheme S2) with sodium chloroacetate was performed following the procedure of Brunsen *et al.*[2] The degree of substitution was determined by ^{1}H NMR as 0.21 (Fig. S2).
^{1}H NMR (400 MHz, D_2O), δ ppm: 5.34, 5.19, 4.99 (anomeric CH, 1H); 4.24, 4.21 (-O-CH_2-COO, 2H); 4.15-3.45 (6H, m).
^{13}C NMR (100 MHz, D_2O), δ ppm: 180.63 (COOH), 100.61 (C1), 82.68, 76.29 (C3), 74.30 (C2), 73.08 (C5), 72.41 (C4), 68.41 (C6).

Dextran-SH



Scheme S3. Preparation of thiol-modified dextran.

The thiol derivatized dextran (DEX-SH, MW = 20 kDa) was synthesized as shown in Scheme S3. First, Cysteamine hydrochloride was coupled to carboxymethyl dextran (CM-DEX) in water using EDC-NHS coupling. Typically, 5 g of CM-DEX was dissolved in 50 mL of demineralized water and cysteamine hydrochloride (1.0 g, 8.8 mmol), EDC (1.5 g, 7.8 mmol) and NHS (0.92 g, 8 mmol) were added and stirred overnight at 25°C. The reaction mixture was then transferred to a dialysis bag (MWCO = 6500 Da), dialyzed first against 0.1 M KCl for 48 hrs (3x2L), then demineralized water for 48 hrs (3x2 L) and freeze-dried. The degree of substitution with SH was 0.10 as determined by ^{1}H NMR (Fig. S3). Yield 4-4.5 g.

^{1}H NMR (400 MHz, D_2O), δ ppm: 5.34, 5.19, 5.00 (1H, anomeric CH), 4.23, 4.19 (2H, O-$\underline{CH_2}$-CO-), 4.15-3.45 (6H, m), 3.26, 3.21 (2H, -$\underline{CH_2}$-SH).
^{13}C NMR (100 MHz, D_2O), δ ppm: 181.67, 180.53 (COOH), 100.60 (C1), 82.70, 76.3 (C3), 74.31 (C2), 73.08 (C5), 72.43 (C4), 68.41 (C6), 45.70 (-C-NH-).

NMR spectra of modified dextrans

All spectra were recorded using an Agilent-400 MR DD2 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, in D_2O.

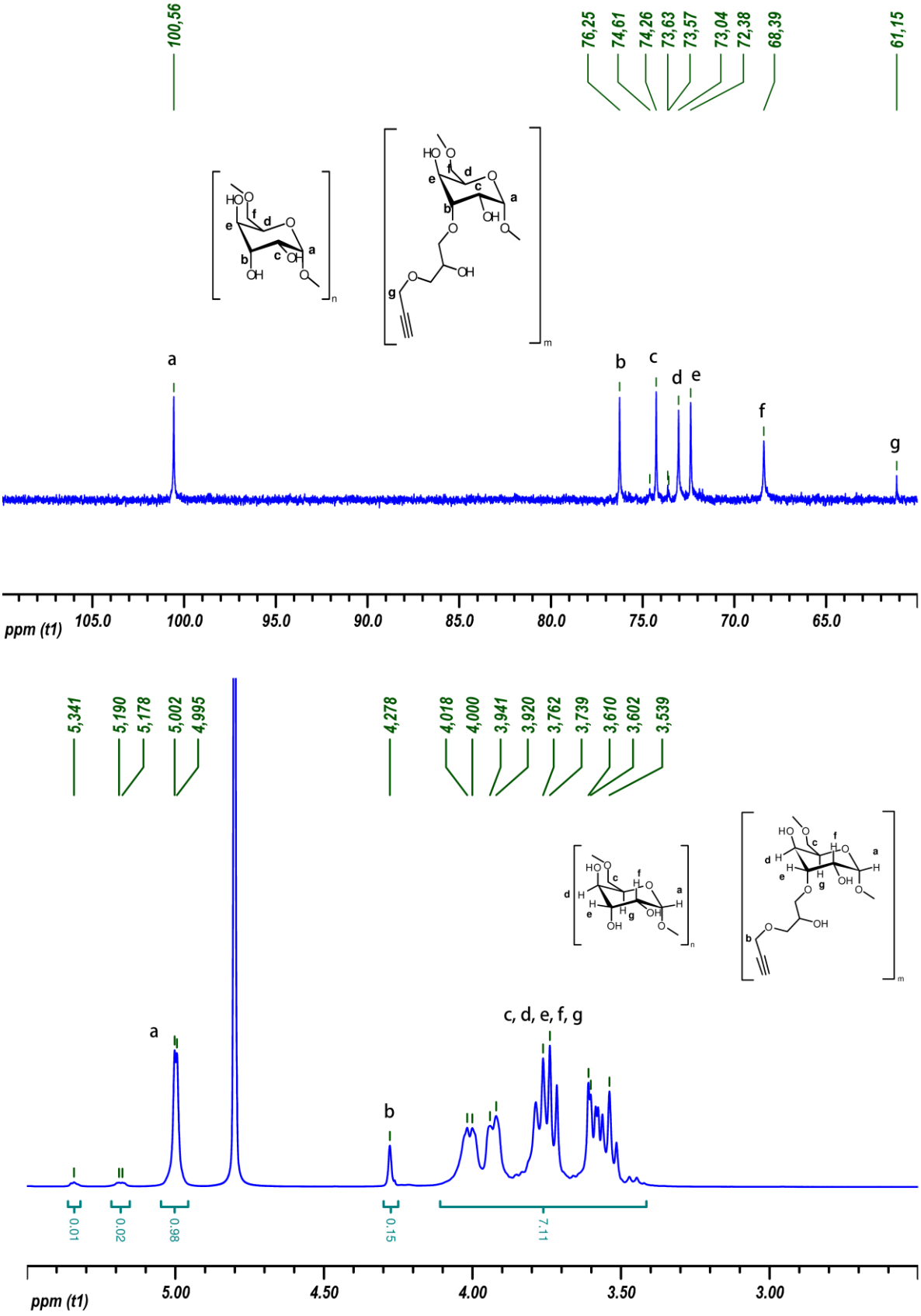


Fig. S1. ^{1}H and ^{13}C NMR spectra of Dextran-GPE.

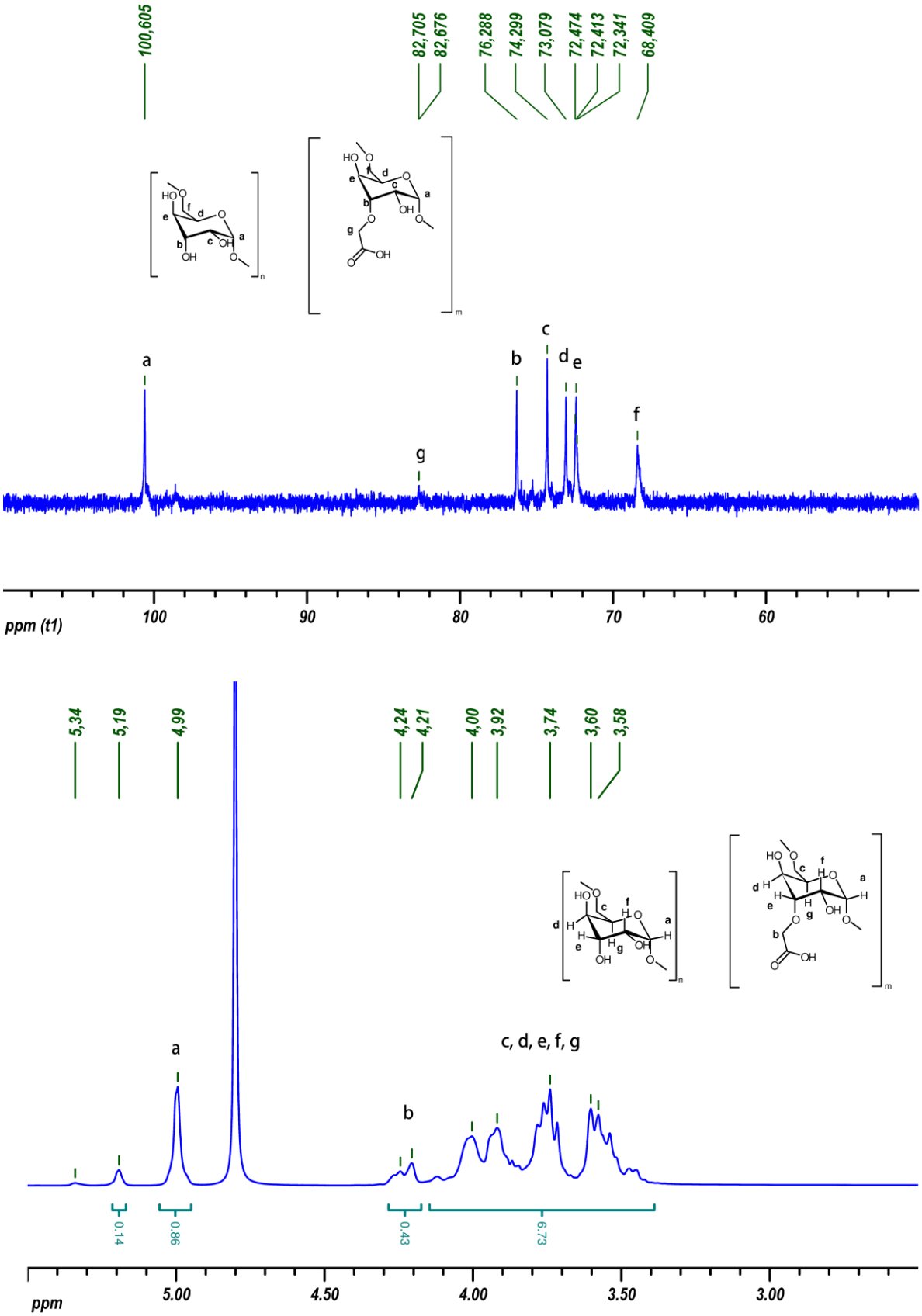


Fig. S2. ^{1}H and ^{13}C NMR spectra of CM-Dextran.

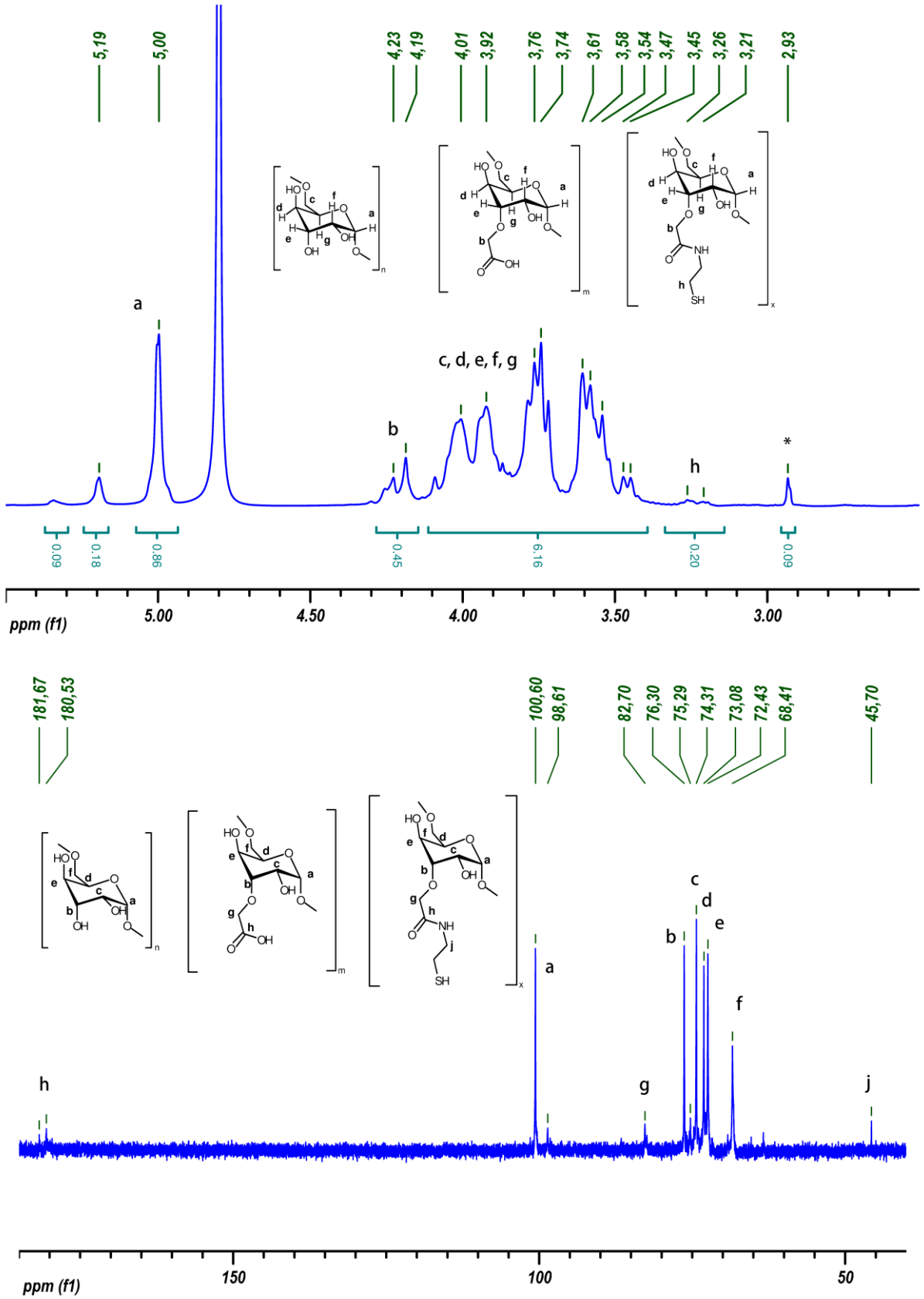


Fig. S3. ^{1}H NMR spectrum of Dextran-SH (* - residual coupling agent (EDC), less than1.5 mol %) and ^{13}C NMR spectrum of Dextran-SH.

Molecular weight estimation of modified dextrans

Molecular weight of modified dextrans used in this study was estimated via gel permeation chromatography (GPC) using Shimadzu Prominence GPC system equipped with with 2x PL aquagel-OH MIXED H columns (Agilent, 8 µm, 300x7.5 mm) and refractive index detector (RID). 10 µL of 5 mg/ml polymers solutions were injected and eluted with a buffer system (0.01 M phosphate buffer, pH =7 + 0.0027 M KCl + 0.13 M NaCl) at 1ml/min and 40°C for 30 minutes. This particular eluent composition was reported suitable for the analysis of CM-dextrans by Brunsen *et al* [2], and is adjusted to screen the charges of carboxylate, which otherwise leads to significant swelling of the polymers coils and subsequent increase of their apparent molecular weight.

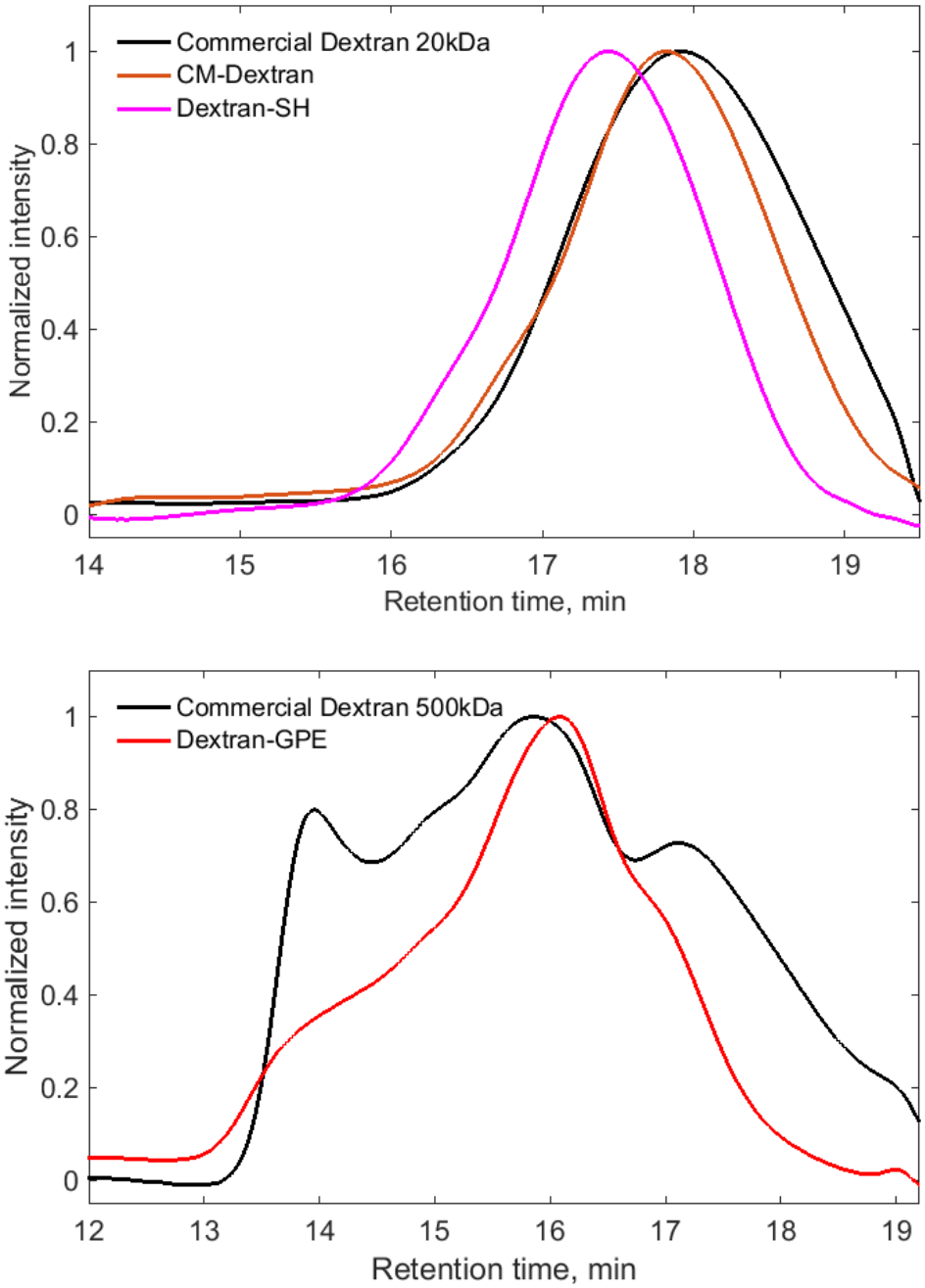


Fig. S4. Molecular weight comparison of modified dextrans with starting polymers.

As can be seen from Fig. S4 modification of dextrans did not lead to significant changes in their molecular weights or polydispersity, which suggests that no chain fragmentation has occurred. In case of modification of dextran 20 kDa, the molecular weight increases slightly, as could be expected. In case of dextran-GPE (MW = 500 kDa), the peak is more symmetrical compared to commercial starting material, potentially due to removal of lower molecular weight fraction during purification by precipitation.

Characterization of the diameter of droplets and particles

Droplets and particles were imaged using optical transmission microscopy. Due to the low contrast of the hydrogel particles on resulting images, particle diameters were determined manually. For each set, the diameter of 250-400 droplets or particles was measured. The resulting data was plotted as histograms and fitted to a normal distribution function. A typical example of the distribution of core and shell diameters of the droplets at optimal particle production conditions is shown in Fig. S5. Values are reported in the main paper as the mean plus/minus two times the standard deviation, $\mu \pm 2\sigma$, which captures over 95% of the droplets or particles.

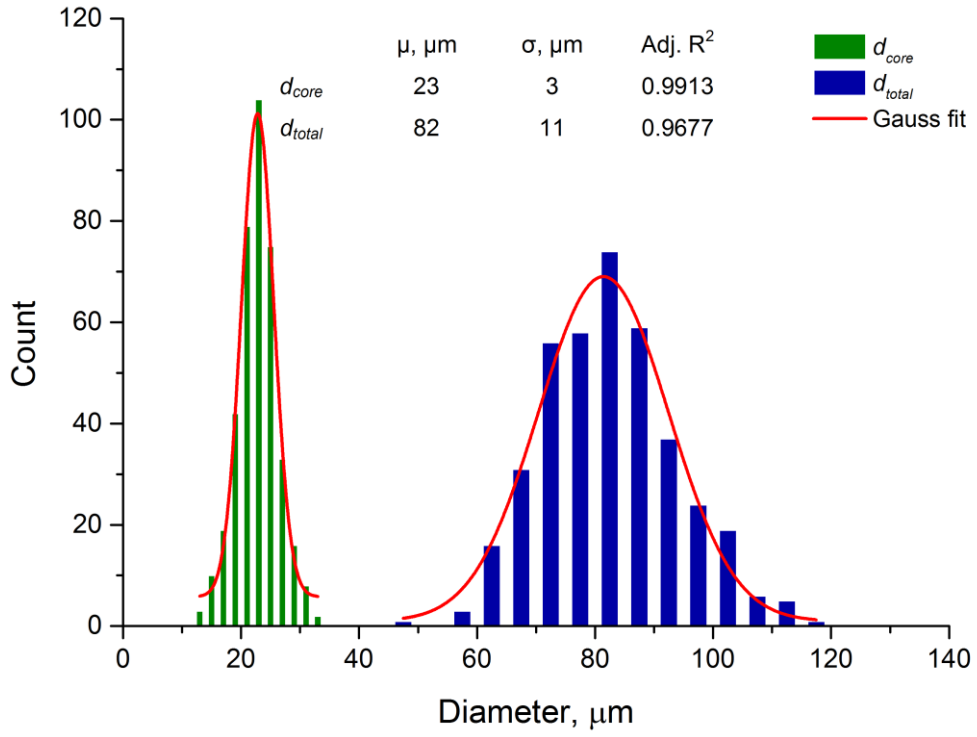


Fig. S5. Histograms of the core diameter and total diameter of the droplets at irradiation point.

Variation of shell to core ratio

We investigated the relationship between volumetric flowrates of shell and core phases (Q_{shell} and Q_{core} respectively) and the size of shells and cores of resulting droplets. Outer phase flowrate (Q_{out}) was fixed at 3.5 µl/min and $Q_{shell} + Q_{core}$ at 0.40 µl/min, while their ratio was varied in range between 3/1 and 1/3. Collected data is represented below.

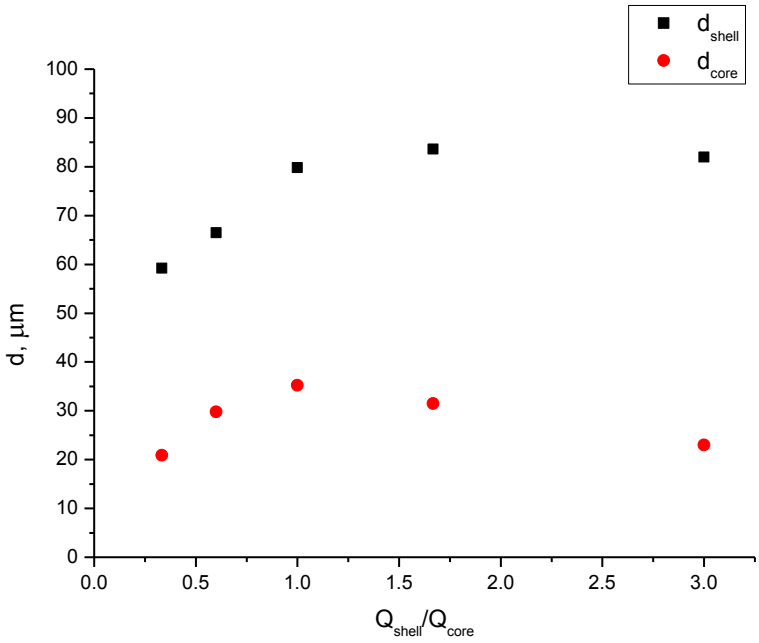


Fig. S6. Dependence of droplets' shell and core mean diameters on the ratio of shell to core flowrates.

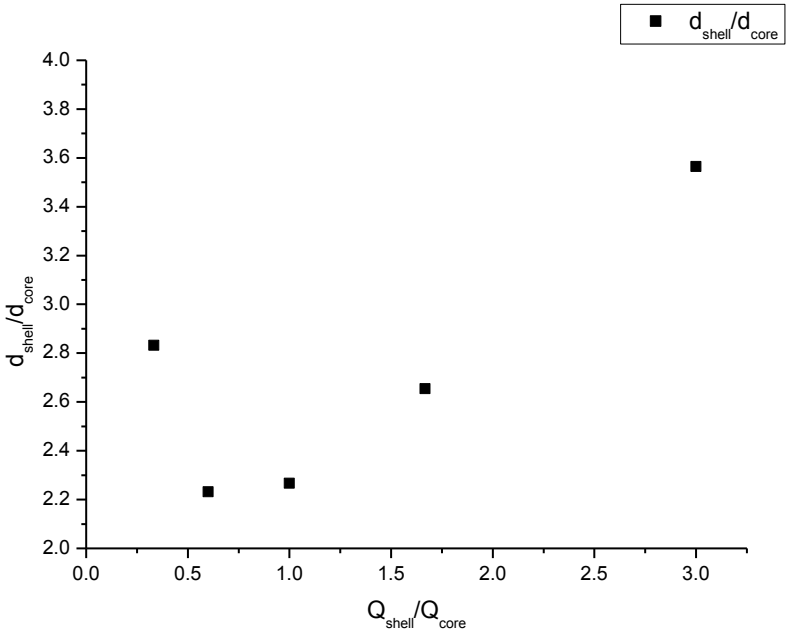


Fig. S7. Dependence of the ratio of droplets' shell to core mean diameters on the ratio of shell to core flowrates.

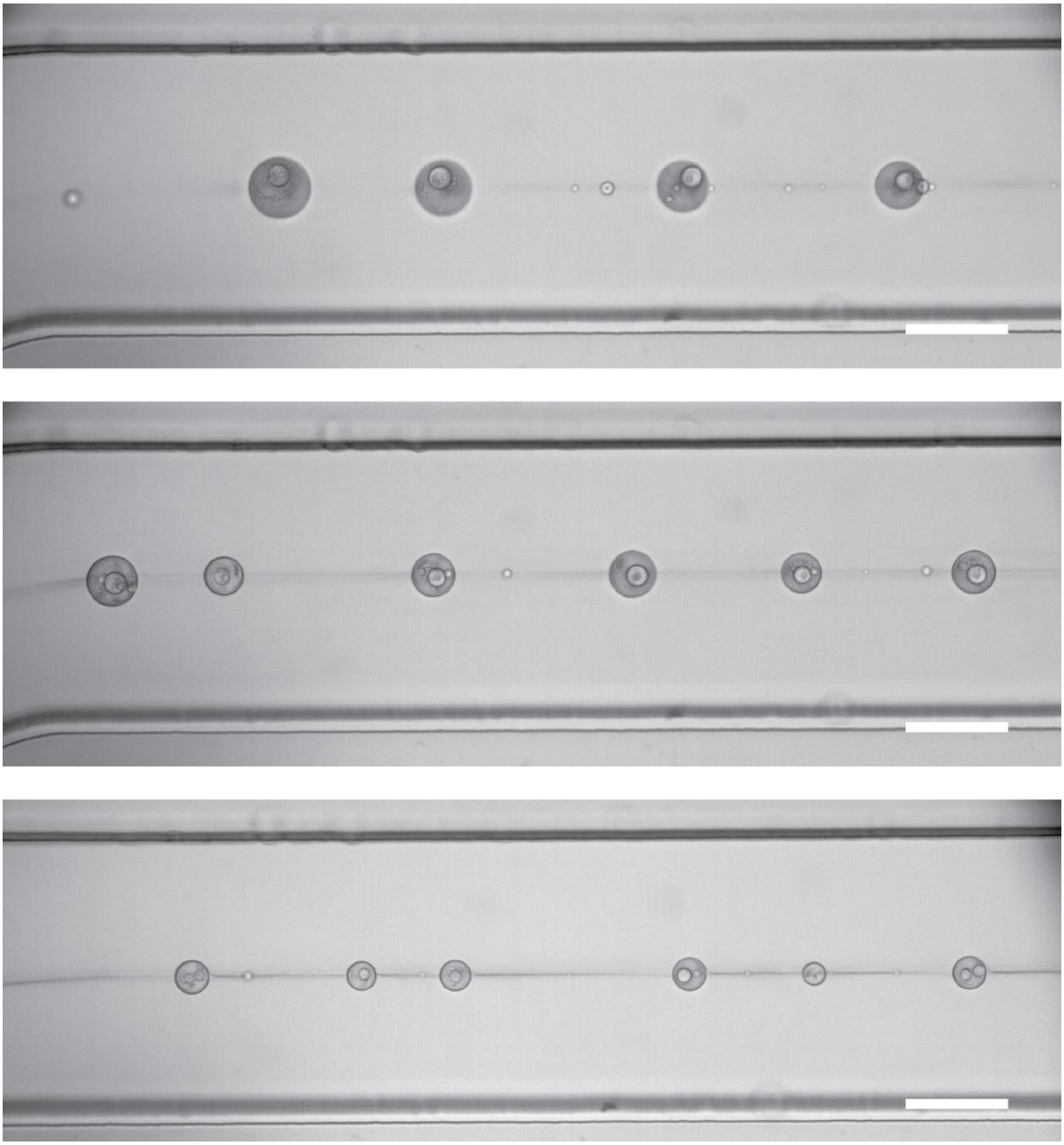


Fig. S8. Representative images of the droplets generated at different ratios of shell to core flowrates. Top to bottom: Q_{shell}/Q_{core} = 3/1, 1/1 , 1/3.

References

1. Nielsen, T. T., Wintgens, V., Amiel, C., Wimmer, R. & Larsen, K. L. Facile synthesis of beta-cyclodextrin-dextran polymers by 'click' chemistry. *Biomacromolecules* **11,** 1710–5 (2010).
2. Brunsen, A. Ritz, U., Mateescu, A., Höfer, I., Frank, P., Menges, B., Hofmann, A., Rommens, P. M., Knoll, W. & Jonas, U. Photocrosslinkable dextran hydrogel films as substrates for osteoblast and endothelial cell growth. *J. Mater. Chem.* **22,** 19590 (2012).