Supporting Information

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Achieving Microparticles with Cell-Instructive Surface Chemistry by Using Tunable Co-Polymer Surfactants

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Supporting Information

Microparticles with defined comb-graft surface chemistry prepared by combining the molecular design of comb-graft surfactants and droplet microfluidics

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Figure S1: Data showing the attachment of *P. aeruginosa* to copolymers including mPEGMA$_{300}$, (a) phenyl methacrylate (PhMA) (b) butyl acrylate (BuA) (c) ethyl acrylate (EA) (d) furfuryl methacrylate (FuMA) (e) isobutyl acrylate (iBuA) (f) lauryl acrylate (LaA) (g) tetrahydrofurfuryl acrylate (THFuA) (h) isobornyl methacrylate (IBMA) (i) 2-N-morpholinoethyl methacrylate (NMEMA) and (j) Phenyl acrylate (PhA). Data points are averaged across n = 6, N= 2 and the standard deviation plotted. Data points with fluorescence intensity = 0 are below the limit of detection.
Figure S2: $^1$H-NMR of the crude end point and pure EGDPEA-co-PEGMA360 surfactant. In the end-point spectra the % conversion was calculated considering one peak related to the monomer and one to the polymer. The actual monomer ratio was calculated from the spectra of the pure material. The multiplet at 5.6 ppm was selected as the relative reference for the spectra. The integral of these peaks was used to assess the EGDPEA contribution to the methylene ester group in the (c+c‘) peak related to the rest of the polymer. This assumption was made as a, b and c have the same number of protons in the EGDPEA structure (two protons). As a result, the assessed value was subsequently divided by the total integral value at 4.15 ppm to evaluate the real influence of this monomer on the copolymer. The same procedure was adopted for EGDPEA-co-PEGMA 500.
Figure S3: $^1$H-NMR of the crude end point and pure EGDPEA-co-mPEGMA surfactant. In the end-point spectra the % conversion was calculated considering one peak related to the monomer and to the polymer. The actual monomer ratio was calculated from the spectra of the pure material. The multiplet at 5.6 ppm was selected as the relative reference for the spectra. The integral of these peaks was used to assess the EGDPEA contribution on the methylene ester group in the (c+c’) peak related to the rest of the polymer. This assumption was made as a,b and c have the same number of protons in the EGDPEA structure (two protons). As a result, the assessed value was subsequently divided by the total integral value at 4.15 ppm to evaluate the real influence of this monomer in the copolymer. The same procedure was adopted for EGDPEA-co-mPEGMA 500.
Figure S4: $^1$H-NMR of the crude end point and pure HPhOPA-co-mPEGMA surfactant. In the end-point spectra the % conversion was calculated considering one peak related to the monomer and to the polymer. The final composition of the co-polymer was defined from the formula in accordance with Izunobi et al.\textsuperscript{[32]}
Figure S5: SEM of polymer microparticles produced using surfactants with different hydrophilic PEGMA/mPEGMA chains (i) showing graphically the sizes of the images A-D (A) Monodisperse particles made from surfactant EGDPEA-co-mPEGMA300 and a HMDA core with size 64.30 ± 1.33 μm (CV = 2.1 %) (B) Monodisperse particles made from surfactant EGDPEA-co-mPEGMA500 and a HMDA core with size 57.81 ± 0.77 μm (CV = 1.3 %) (C) Monodisperse particles made from surfactant EGDPEA-co-PEGMA360 and a HMDA core with size 65.24 ± 2.74 μm (CV = 4.2 %) (D) Monodisperse particles made from surfactant PVA and a HMDA core with size 61.60 ± 2.93 μm (CV = 4.8 %)
Figure S6: Flow diagram of oil-in-water microfluidics system with HMDA core material and HPhOPA-co-mPEGMA<sub>300</sub>. ○ denotes idealistic dripping behaviour, × denotes jetting behaviour, △ denotes formation of satellite droplets, Δ denotes flow rates which caused wall wetting events and ♦ denotes flow rates that cause unstable large droplet formation. Blue denotes region of flow rates that produces monodisperse emulsions, red denotes areas of no emulsion formation and orange denotes area which produces variable emulsion sizes. Images show examples of dripping, jetting, satellite droplet formation and wall wetting events respectively.
Figure S7: $^1$H NMR spectrum showing minimal amount of leaching from particles when exposed to deuterated chloroform. Internal control used is TMS. Peaks in expanded section refer to acrylate bond protons, which facilitated calculation of the concentration of HMDA (0.5 mM).

As a test of microparticle cytotoxicity, a cell viability test was performed on mammalian cells, specifically MRC-5 (ATCC® CCL171™) human lung fibroblasts. Particles were made with 2 % (w/v) EGDPEA-co-mPEGMA surfactant but with varying photoinitiator to determine the effect on cell viability (Figure S8).
Figure S8: As a measure of cytotoxicity, a cell viability study using MRC-5 (ATCC® CCL171™) human lung fibroblast cells. Particles produced with different photoinitiator content up to 5 wt % were used across a range of different particle concentrations. Cells grown on tissue culture plastic (TCP) and cells treated with Triton-X were used as positive and negative controls respectively. Measurements taken over N = 3 biological repeats.

As shown in Figure S8, cell viability was maintained at above 80 % up to 5 mg/ml of microparticles for both 1 and 2 wt % photoinitiator, showing that the particles do not cause cell toxicity. However, at all photoinitiator concentrations and 10 mg/ml cytotoxicity is observed. This is attributed to the high particle density covering the cell layer and blocking the flow of growth medium, causing cell death.
Figure S9: (a) Surface coverage by *P. aeruginosa* biofilms quantified after 24 h incubation in RPMI on an EGDPEA or HPhOPA homopolymer film respectively or on glass. Error bars equal ± 1 SD unit, n = 3. Film data were normalised to a glass control for comparison (b) The corresponding confocal microscopy images for mCherry tagged *P. aeruginosa* growing on each polymer surface and glass control. Each image is 568 x 568 µm.
Figure S10: Correlation between pro- and anti-attachment on 2D flat surface and 3D Microparticles and 3D controls. The quantification of surface coverage by single species (P. aeruginosa) biofilms after 24 h incubation in RPMI. Error bars equal ± 1 SD unit, n = 3. The confocal microscopy images for mcherry tagged P. aeruginosa growing on each polymer surface are 295 x 295 µm and 568 x 568 µm for the MPs and films, respectively.