Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

On-the-fly Exchangeable Microfluidic Nozzles for Facile Production of Various Monodisperse Micromaterials

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Experimental Section

Materials: Polymethylmethacrylate (PMMA) and O-rings (Viton, #1: inner diameter 0.74 mm, outer diameter 2.78 mm, #2: inner diameter 1.07 mm, outer diameter 3.61 mm) were provided by the University of Twente. Fluorinated ethylene propylene tubing (FEP, inner diameter 500 μm, outer diameter 1/16") was purchased from IDEX Health & Science. Polyimide-coated fused silica capillaries (TSP type, various inner diameters, outer diameter 360 μm) were purchased from Polymicro Technologies. Borosilicate capillaries (inner diameter 700 μm, outer diameter 870 μm were purchased from CM Scientific. Quick-set epoxy adhesive (RS 850-956) was purchased from RS Components. Polydimethylsiloxane (PDMS, Sylgard 184) was purchased from Dow Corning. Silicone tubing (inner diameter 310 μm, outer diameter 640 μm) was purchased from Helix Medical. Abrasive (Cif) was purchased from Unilever. Fluorinated silane (Aquapel) was purchased from Vulcavite. Hexadecane, Span 80, sodium dodecyl sulphate (SDS), phosphate-buffered saline (PBS), horseradish peroxidase (HRP; type IV), H_2O_2 (with inhibitor), acetic acid, polyethylene glycol diacrylate (PEGDA; 575 Da), 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (I2959), polyethylene glycol (PEG; 12 kDa), $CaCl_2$, and dextran-FITC (2000 kDa) were purchased from Sigma-Aldrich / Merck. Dextran-tyramine (Dex-TA; ~20 kDa) with 15 tyramine moieties per 100 repetitive units was synthesized as previously described.[35] Alginate (80 to 120 cP) and catalase (from bovine liver) were purchased from Wako Chemicals. $CaCO_3$ nanoparticles were purchased from Nanomaterials Technology. Red fluorescent particles (0.6 μm polystyrene Fluoro-Max) were purchased from Thermo Fisher Scientific.

Microfluidic device production and operation: The microfluidic device was designed using SolidWorks software and manufactured by cutting using a belt saw and drilling using a column drill. Drilling was done at a relatively low rotational frequency (<1000 rpm) in the presence of concentrated soap (Dreft, Procter & Gamble) solution to prevent cracking of the PMMA. To

recover transparency, the inside of the device was polished by pulling a knotted cotton thread soaked in abrasive through the center channel. Nozzles were manufactured by hand-cutting fused silica or borosilicate capillaries using a tungsten-carbide glass cutting knife. Optionally, the nozzles were chemically or physically modified as follows. Hydrophobic nozzles were produced by oxygen plasma treatment (PDC-002, Harrick Plasma) for 1 minute at (21% O_2, 30 W, 0.2 mbar) followed by coating with fluorinated silane solution. UV-transparent nozzles were produced by flaming off the fused silica capillary's polyimide coating. Absorbance of 365 nm light was measured using an optical power meter (1916-C with 818-ST-UV sensor, Newport). The nozzles were either glued into tubing using quick-set epoxy adhesive or inserted in tightly fitting silicone tubing that self-sealed to the nozzle through hydrophobic interactions. Multi-bore nozzles were fabricated by gluing multiple fused silica capillaries into a borosilicate capillary using PDMS. Tubing and nozzles were sealed to the microfluidic device using tight-fitting O-rings. Tubing was connected to syringes (Gastight, Hamilton) that were controlled by low-pressure syringe pumps (neMESYS, Cetoni). On-the-fly nozzle exchange was performed by sequentially pausing the syringe pump, pulling out the original nozzle, inserting a distinct nozzle, starting the syringe pump, and recalibrating the flow rate to obtain a Capillary number at which stable droplet production was achieved. For droplet production, typical dispersed flow rates for the 75, 200, and 700 μm nozzles were 5, 30, and 200 μl/min, respectively. Total flow rates can be calculated from the capillary numbers as plotted in Figure 3.

Micromaterial production: To produce microemulsions, water or water with 5% (w/v) Dex-TA was emulsified in hexadecane with 1% (w/v) Span 80 using a pristine or hydrophobic fused silica nozzle in T-junction, coaxial flow, or flow focusing configuration. Alternatively, hexadecane was emulsified in water with 1% (w/v) SDS using a pristine or hydrophobic fused silica nozzle in flow focusing configuration. To produce all-aqueous two-phase flows, water with 1% (w/v) alginate was injected in water with 30% (w/v) PEG using a combination of a

pristine fused silica nozzle (inlet) in combination with a borosilicate glass nozzle in coaxial flow and flow focusing configuration, respectively. To produce solid Dex-TA microspheres, 5% (w/v) Dex-TA and 25 U/ml HRP in PBS was emulsified in hexadecane with 1% (w/v) Span 80 using a hydrophobic fused silica nozzle in flow focusing configuration connected to a silicone tubing. The silicone tubing was submerged in 30% (w/w) H_2O_2, which diffused through the silicone and the oil phase into the Dex-TA precursor microemulsion, thereby inducing the enzymatic crosslinking of the tyramine-conjugated polymer to yield Dex-TA microspheres, as previously described.[19] To produce core-shell Dex-TA microspheres, the protocol for solid Dex-TA microspheres was used, but with addition of 83000 U/ml catalase to the hydrogel precursor solution. To produce PEGDA microspheres, 25% (v/v) PEGDA and 0.25% (w/v) I2959 in water was emulsified in hexadecane with 1% (w/v) Span 80 using a hydrophobic polyimide-coated fused silica nozzle in flow focusing configuration connected to a silicone tubing. The microfluidic device was placed under a UV light source (365 nm, ~50 mW/cm^2; LC8 Lightningcure L9588, Hamamatsu), enabling the on-chip photocrosslinking of the microemulsion into PEGDA microspheres. Emulsions were broken by multiple hexadecane washes (i.e., to reduce the concentration of Span 80 surfactant) in the presence of water or PBS. To produce alginate microparticles, a solution of 0.5% (w/v) alginate and 1 g/l $CaCO_3$ in water was emulsified in a 50/50 hexadecane/mineral oil mixture with 1% (w/v) Span 80, and subsequently injected in the same oil solution supplemented with 2 µl/ml acetic acid using a combination of a pristine fused silica nozzle and a hydrophobic fused silica nozzle in coaxial and flow focusing configuration. Diffusion of the acid through the intermediate oil flow enabled controlled dissolution of the $CaCO_3$ into Ca^{2+} that induced the ionic crosslinking of alginate. To produce alginate microfibers, water with 1% (w/v) alginate was injected in water with 30% (w/v) PEG, which was focused by a flow of water with 30% (w/v) PEG and 100 mM $CaCl_2$ using a combination of a pristine fused silica nozzle (inlet) in combination with a borosilicate

glass nozzle in coaxial flow and flow focusing configuration, respectively. Diffusion of the Ca^{2+} ions through the intermediate PEG flow enabled controlled ionic crosslinking of the alginate, resulting in smooth alginate microfibers. Replacing the single-core inlet nozzle by a multi-core inlet nozzle enabled the formation of a multimaterial Janus type alginate microfiber.

Staining, visualization, and image analysis: On-chip multiphase flows were visualized using a stereomicroscope setup (Nikon SMZ800 equipped with Leica DFC300 FX camera). Collected micromaterials were imaged using standard phase contrast microscopy. Alginate microfibers were stained by adding fluorescent particles and/or dextran-FITC and visualized using fluorescence microscopy (EVOS FL, Thermo Fisher Scientific). Size distribution analysis of droplets and particles was done using ImageJ software. The droplet production rate ($f_{droplet}$) in Hz was determined from a microscopic timelapse recording of the droplet generator.

Statistics: All droplet diameters were determined from at least 30, 30, and 5 samples for the 75 µm, 200 µm, and 700 µm nozzles, respectively, and were reported as the average ± standard deviation or as a histogram with the coefficient of variation (CV = standard deviation / average). The relative transmissions in Figure 4e and Figure S1 were determined from at least three measurements and reported as the average ± standard deviation. The droplet frequencies in Figure 4g for t = 0-30 s were determined from 3 samples and for t = 30-300 s from one sample, and (where possible) reported as the average ± standard deviation. All micromaterials diameters were determined based on at least 12 samples and reported as a histogram with the CV. Significance was determined based on one-way Anova analysis. All linear regression and statistical analyses were performed in OriginPro2016.

Schematics: All graphs were made using OriginPro 2016 software. All schematics were made using Solid Works 2017, Adobe Illustrator CS6, and Microsoft PowerPoint software CorelDRAW X7 software.

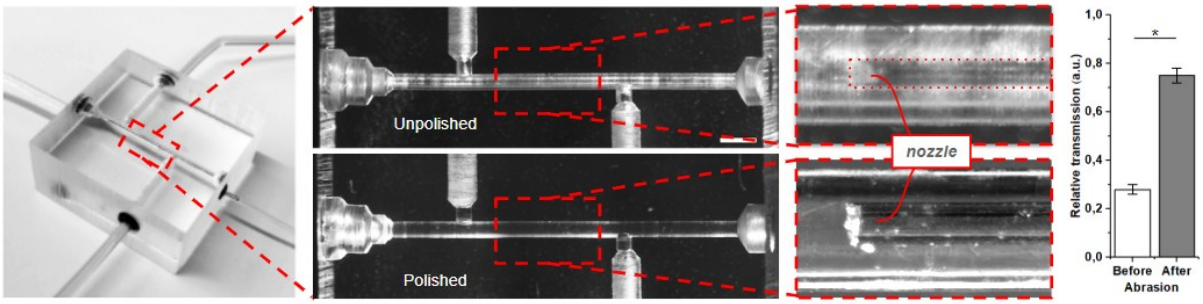


Figure S1. Polished versus unpolished microfluidic device channels. Polishing the PMMA based microfluidic device significantly increased transparency, thereby enabling on-chip monitoring of the nozzle. * indicates significance with $p<0.0001$.

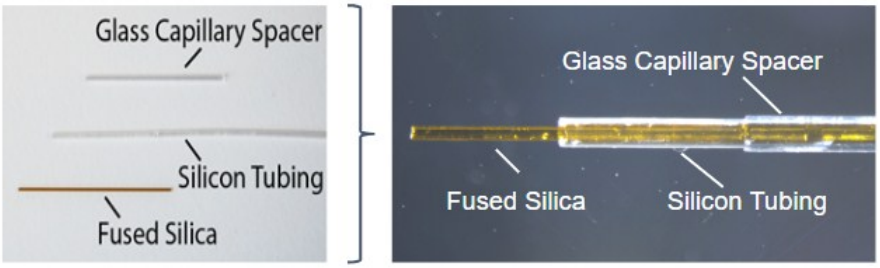


Figure S2. Exchangeable nozzle assembly. To assemble an exchangeable nozzle, a fused silica capillary was inserted into a silicone tubing, which was inserted into a glass capillary spacer.

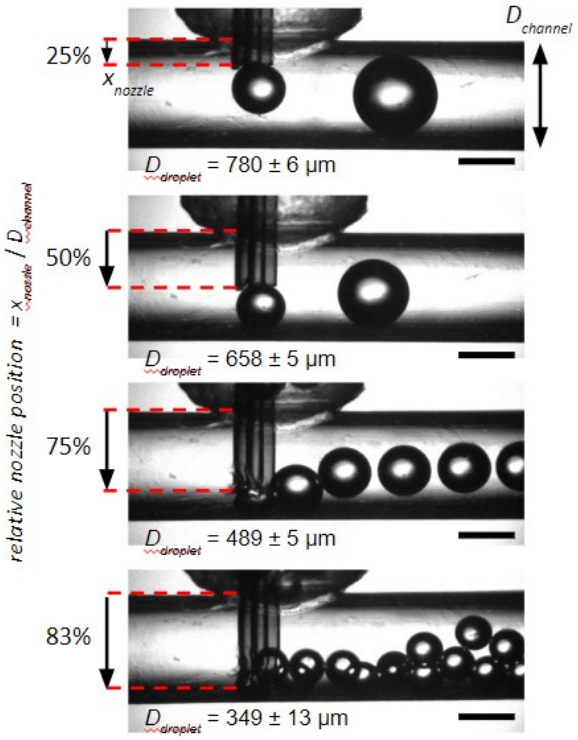


Figure S3. On-the-fly fine-tuning of nozzle position controls droplet size. All microphotographs were taken during emulsification of water (10 µl/min) in hexadecane with 1% Span 80 (100 µl/min) using a 150 µm nozzle in T-junction configuration. The size of droplets was largely determined by the position of the nozzle within the channel. On-the-fly nozzle repositioning within a transparent microfluidic device thus enables the swift iteration towards a functional droplet generator that operates within the desired production regime. Scale bars indicate 500 µm.

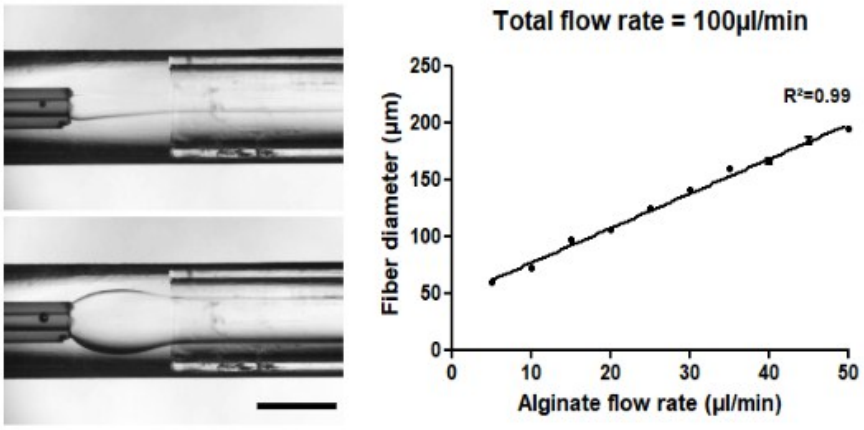


Figure S4. All aqueous two-phase flow generation control. The diameter of alginate flow and resulting microfibers could be linearly ($R^2 = 0.99$) controlled by the ratio of alginate to the total flow rate. Scale bar indicates 700 µm.

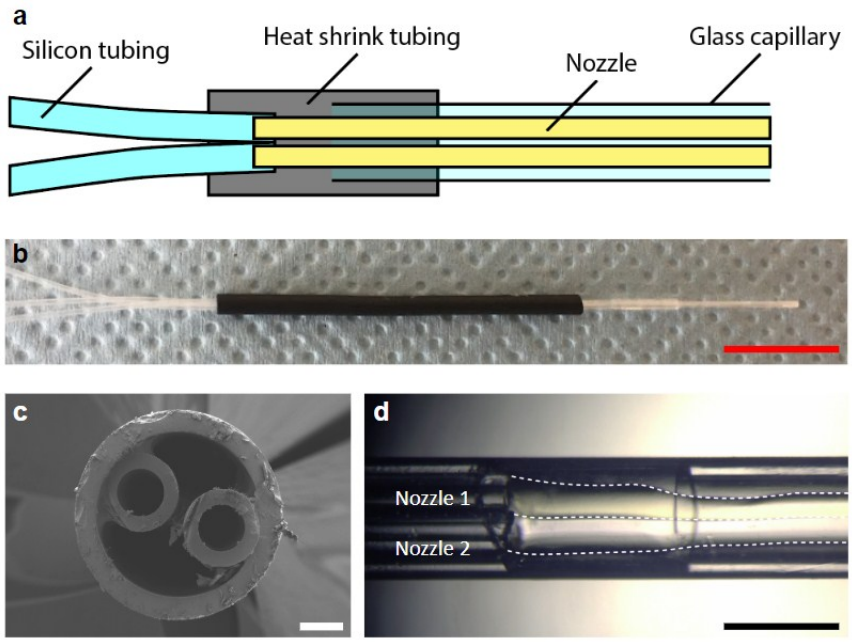
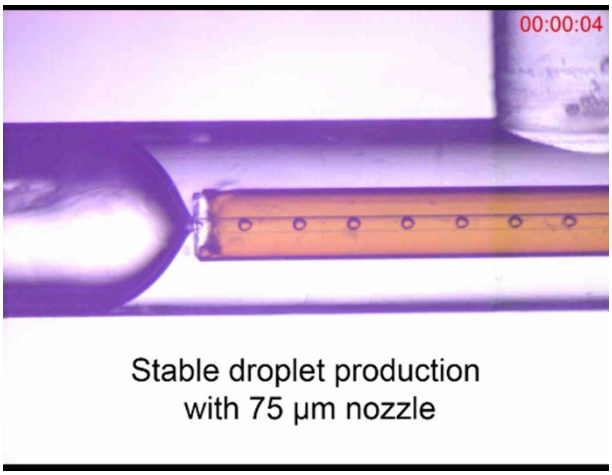


Figure S5. Assembly and operation of multibore nozzle for the production of Janus micromaterials. (**a**) Schematic overview, (**b**) photograph, and (**c**) scanning electron microscopy image of the multibore nozzle assembly that was used to generate (**d**) focused multiphase aqueous co-flows for the production of Janus type microfibers. Red scale bar indicates 1 cm. White scale bar indicates 200 µm. Black scale bar indicates 1 mm.



Movie S1 (still). On-the-fly nozzle exchange allows for a switch in droplet size production regime within 1 minute. t = 0-10s: droplets are produced using a nozzle with a diameter of 200 µm. t = 10s: flow is paused. t = 13s: 200 µm diameter nozzle is retracted. t = 25s: nozzle with a diameter of 75 µm is inserted. t = 35s: flow is restarted. t = 40-50s: flow rates are adjusted. t = 52s: stable droplet production achieved with 75 µm nozzle.