Electronic Supplementary Material (ESI) for Chemical Communications

Lab-chip HPLC with integrated droplet-based microfluidics for separation and high frequency compartmentalisation

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Supplementary Information

Chemicals and Materials

TPE (CFS Fiberglass, UK) was prepared according to the manufacturer's instructions by mixing with its polymerisation catalyst, methyl ethyl ketone peroxide (MEKP). Polyethylene terephthalate (PET) (Daedong Polymer, South Korea) was used as a base substrate. 5μm C18 (Octadecyl silane) particles were purchased in bulk from Phenomenex, USA and used as the chromatographic stationary phase. Alexa fluor 488 (Invitrogen, USA) and fluorescein isothiocyanate (Sigma-Aldrich, USA) were chosen as analytes. The continuous phase used in droplet formation was an 10 % (v/v) mixture of FC-70 (3M Fluorinert, USA) and 1H, 1H, 2H, 2H-perfluorooctanol (PFO, Sigma-Aldrich).

Fabrication of the hybrid particulate LC - droplet based microfluidic device

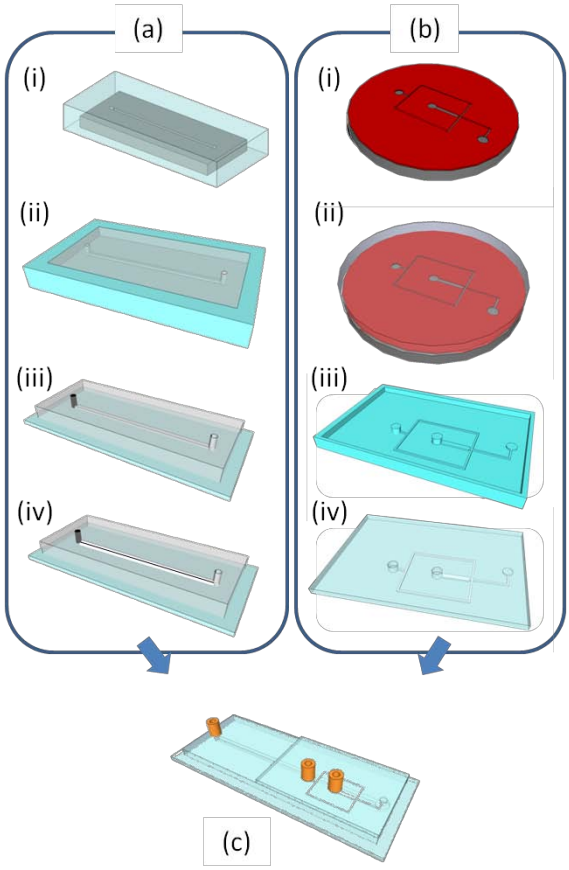

Figure S1. Schematic of the hybrid device fabrication, (a) LC separation channel in the first layer (i) PDMS casting form the master mould (ii) separation of the PDMS mould, TPE pouring then semi-curing at 60 °C (iii) bonding on PET substrate and full-curing at 76 °C (iv) packing the channel by C18 beads, (b) droplet generation channel in the second layer (i) SU-8 master mould (ii) PDMS casting (iii)

removing PDMS mould, TPE pouring and semi-curing at 60 °C (iv) peeling off, (c) bonding the two channels, PEEK attachment and full-curing at 60 °C

Figure S1 shows the steps in fabricating the complete microfluidic device. The procedure begins with making the separation channel in a first layer (Figure S1(a)). The master mould (2 cm (W) x 0.4 cm (H) x 5 cm (L)) was produced using a microstereolithography system (Envision TEC, UK) and consists of a 1 mm x 1 mm x 50 mm channel which was then cast in PDMS. The resulting PDMS mould (cured at 65 °C for 4h) was peeled off and used as a practical mould for TPE casting. TPE resin and the MEKP catalyst were mixed in a ratio of 100 : 1 (w/w), degassed and decanted onto the PDMS mould. The resulting structure was partially cured in an oven for 10 min at 60 °C. In the meantime, the PET substrate was sonicated in isopropyl alcohol (IPA) and dried with N_2 gas. Subsequently the surface of the PET substrate was treated with an O_2 plasma at 70 mW for 12 sec. The semi-cured TPE channel was carefully separated from the mould and a stainless steel HPLC frit (Phenomenex, USA) with a 2 µm pore size was inserted at the outlet void in the channel. The channel/frit assembly was then contacted to the PET substrate. Finally, the entire device was heated at 76 °C for 1 hr to complete the TPE cure and then cooled down to room temperature over several minutes. To pack the TPE separation channel, a slurry of 5 µm C18 beads (2 mg in 1ml of methanol) was introduced into the channel by vacuum through the frit. After fabrication of the separation channel, the droplet channel was bonded to the separation channel.

Unlike the separation channel, the TPE droplet channel was fabricated using a SU-8 patterned master via standard photolithography. A 250 µm thick SU-8 photoresist was coated on the silicon wafer and exposed to UV radiation through a negative patterned film mask. After development, the PDMS mould was cast and carefully peeled off the SU-8 mould. The centre of the PDMS mould was surrounded with 2 mm thick PDMS walls to define the final outside dimension of the device and two PDMS pillars were used to define the outlet and oil inlet. The semi-cured TPE droplet channel was prepared from the PDMS mould using the procedure described above using a 15 min semi-curing time. The semi-cured TPE droplet channel was separated from the PDMS mould and contacted with the TPE separation channel, aligning the inlet of the droplet channel to the outlet of the separation channel. Finally, PEEK unions (Phenomenex, USA) were attached to the inlets and outlets using epoxy and the device was fully cured at 60 °C for 1 hr.

Figure S2. The fabricated hybrid HPLC/droplet-based microfluidic device

The regeneration of the C18 column and the surface treatment of the TPE droplet channel

The C18 column in the channel can dry out during the fabrication of the droplet channel and attachment of the PEEK unions and may need regeneration before the use. In addition, to generate stable water-based aqueous droplets in oil, the surface of the droplet channel should be hydrophobic. Accordingly, a fluorinated solvent (Aquapel, PPG Industries, USA), was introduced into the droplet channel at 10 µl/min for 1 min. To prevent solvent from entering the column, DI water was pumped into the column and stopped when it reached to the outlet of the separation channel. Using this procedure, the surface of the droplet channel was rendered hydrophobic without affecting the separation column. For column regeneration, ten column volumes of cleaning solvent (50 % : 50 % (v/v) methanol : DI water) was pumped through the column immediately following the hydrophobic treatment of the droplet channel.

RP-HPLC separation and LIF detection

26 mM phosphate buffer (pH 7) was prepared as a mobile phase by mixing sodium phosphate and sodium hydroxide in DI water. This was mixed with UV-grade methanol (HiPerSolve for HPLC, BDH Prolabo) in a ratio of 5 % : 95 % (v/v) methanol : buffer and filtered through a 0.02 µm membrane filter. The mobile phase was pumped into the channels at 50 µl/min by an Agilent HP 1050 HPLC pump. Oil was introduced using a precision syringe pump (Harvard Apparatus, USA) at volumetric flow rates of 50, 75 and 100 µl/min. Alexa fluor 488 and fluorescein isothiocyanate (FITC) were diluted in the mobile phase to final concentrations of 0.02 µM and 50 µM. Injection volumes were 5 µl.

LIF was used for detection of both the continuous column effluent and the compartmentalisation into droplets. The 488 nm line from an Ar+ laser was focused into the microchannel to excite the dyes using a 10 x 0.3 NA objective lens. Fluorescence photons were recorded as a function of time using an avalanche photodiode (AQR-141, EG&G, Perkin-Elmer) with 50 µs sampling rate for 700 s.

Additional results

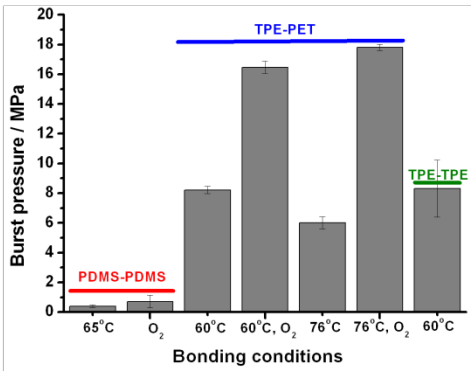

Figure S3. Burst pressure test as a function of bonding conditions and device materials

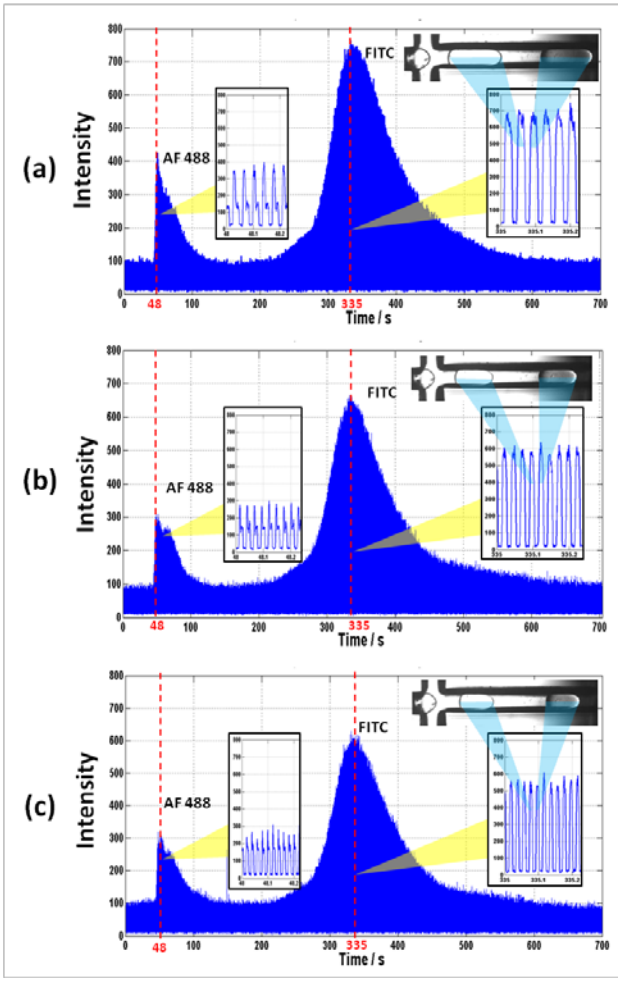

Figure S4. Chromatograms of separated dyes (AF488 and FITC) after compartmentalisation with(a) 27 Hz droplet generation (50 µl/min running buffer-50 µl/min oil) (b) 39 Hz droplet generation (50 µl/min running buffer-75 µl/min oil) and (c) 50 Hz droplet generation (50 µl/min running buffer-100 µl/min oil)

Run	N		R_s
	AF 488	FITC	AF 488 – FITC
Continuous flow	19.47	63.77	2.58
Compartmentalisation with 27 Hz	19.47	63.10	2.52
Compartmentalisation with 39 Hz	12.46	78.32	2.55
Compartmentalisation with 50 Hz	13.40	79.15	2.55

Figure S5. The number of theoretical plates (N) and separation resolution (R_s) of the separated dyes (AF 488 and FITC)

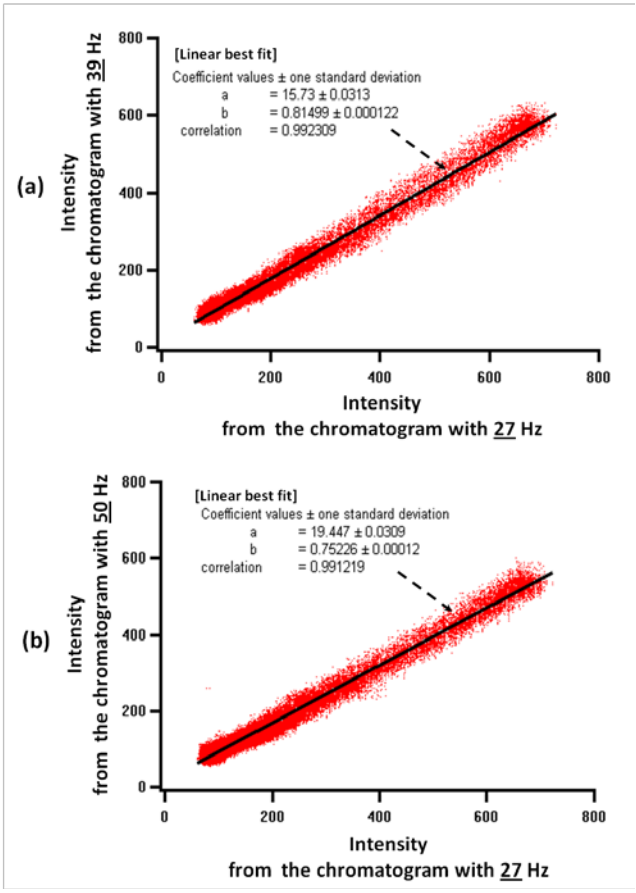

Figure S6. Correlation among chromatograms from compartmentalised flow (a) linear best fit between compartmentalisation with 27 Hz and 39 Hz , (b) linear best fit between compartmentalisation with 27 Hz and 50 Hz