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Rapid Preparation of Highly Reliable PDMS Double Emulsion Microfluidic Devices†

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This article presents a simple and highly reliable method for preparing PDMS microfluidic double emulsion devices that employs a single-step oxidative plasma treatment to both pattern the wettability of microchannels and to bond the chips. As a key component of our strategy we use epoxy glue to define the required hydrophobic zones and then remove this after plasma treatment, but prior to bonding. This novel approach achieves surface modification and device sealing in a single process, which reduces chip preparation times to minutes and eliminates the need for unreliable coating processes. The second key element of our procedure is the maintenance of the patterned surfaces, where we demonstrate that immediate filling of the prepared device with water or the use of solvent-extracted PDMS vastly extends the operational lifetimes of the chips. The reliability of this technique is confirmed by generating water-in-oil-in-water (W/O/W) double emulsion droplets with controlled core/shell structures and volumes, while its versatility is demonstrated by simply using a different placement of the epoxy glue on the same chip design to create oil-in-water-in-oil (O/W/O) double emulsion droplets. Both W/O/W and O/W/O double emulsion droplets can therefore be created from the same soft-lithography mould. This simple method overcomes one of the key problems limiting the wider use of double emulsions – lack of reliability – while its speed and simplicity will facilitate the high-throughput production of monodisperse double emulsions. Our method is demonstrated to produce double emulsion down to 55 µm in diameter and could be readily extended to produce microfluidic chips with more complex hydrophilic and hydrophobic patterns.

Introduction

Double emulsions have enormous potential for a wide range of applications including vesicle preparation,[1] drug delivery,[2] chemical extraction,[3] materials synthesis,[4-8] and the controlled release of encapsulated actives.[9] They can also be analysed in commercial fluorescence-activated cell sorting (FACS) machines, where the requirement for an aqueous carrier fluid makes the technique incompatible with simple water-in-oil single emulsions with this technique.[10,11] The study and use of these fascinating structures has grown rapidly over the last 10 years, largely thanks to the emerging technology of microfluidics,[12] which promises the ability to control the size,[13] the number of internal droplets[14] and the production speed[15] of the emulsion particles. However, despite this promise, microfluidic devices remain under-used for double emulsion production due to the challenges associated with the construction of devices that can provide reproducible and stable double emulsion generation.

A number of strategies have been used to fabricate microfluidic chips for double emulsion generation, where all rely on the ability to selectively control the wettability of the microchannel surfaces. Many devices are constructed from glass capillaries[16] or glass wafers[17] as these can be readily surface-functionalised using self-assembly monolayers (SAM). However, manufacture of the glass capillary devices suffers from the lack of flexible and reproducible methods for capillary alignment, while glass wafers require time-consuming etching to define the channels. The fabrication of microfluidic devices from PDMS offers many advantages, including flexibility, rapid manufacture and low cost. A significant challenge facing the use of PDMS devices, however, is achieving surface modification. A wide range of approaches including UV/ozone treatment,[18,19] layer by layer deposition,[20] chemical grafting using oxygen plasma polymerization,[21,22] and sol-gel methods[23] have been explored, but all require multi-step processing and the use of different solvents can cause contamination. It has also proven very difficult to generate PDMS devices in which the surface functionalisation is spatially-patterned.

The most widely used method for modifying the surface of PDMS is treatment with a direct oxygen plasma; this makes the surface hydrophilic due to oxidation of surface siloxane groups to silanols.[24] Although effective in the short-term, it is difficult to achieve spatial control of the plasma treatment, and hydrophobic recovery can occur within a few hours, rendering the microfluidic chip useless. A recent article used oxygen plasma treatment to spatially pattern the wettability of PDMS double emulsion chips,[25] where the chips were plasma-bonded and then left in air for 48 h to enable hydrophobic recovery before re-exposing specific zones to

the plasma. This was achieved by blocking inlet ports near the desired hydrophobic areas with tape, and employing diffusion barriers to limit diffusion of radicals from treated regions to the unexposed regions. The duration of the plasma oxidation had to be optimised for every chip design to achieve the desired balance of hydrophilicity and hydrophobicity.

This article describes an extremely simple, highly versatile and reliable method that creates PDMS microfluidic chips for double-emulsion production. We again employ an oxygen plasma to create the required spatial patterning of wettability, but now use epoxy glue to demark the desired hydrophobic regions on the channel layer and the partner PDMS backing slab. Surface treatment and chip bonding can therefore be achieved with a single plasma treatment step, enabling chips to be prepared in minutes. Immediate filling of the prepared device with water then significantly retards the hydrophobic recovery process, such that a single device can remain operable for days. We also explored the construction of devices from PDMS that had been solvent-extracted to remove uncured oligomers. This pre-treatment provides even greater surface stability such that devices stored in air were fully functional after 3 days. We also demonstrate the flexibility of our approach by using it to create chips that generate either W/O/W or O/W/O double emulsions from the same initial device design. It is envisaged that this strategy can be readily extended to create alternative chip designs requiring more complex patterns of surface wettability.

Materials and Methods

Materials

PDMS (Sylgard 184 Elastomer Kit) was obtained from Dow Corning, while Millipore water containing 5 vol% of surfactant (polysorbate 80, Alfa Aesar, UK) was employed as the water phase. 2-propanol was used for extracting uncured oligomer of PDMS. Sunflower oil was used as the oil phase. Blue food colouring dye (Knightsbridge PME Ltd., UK) was added to the water at 10 vol% to improve the visualisation of the water phases within the W/O/W and O/W/O emulsions. Rhodamine 6G purchased from Acros Organics (Fisher Scientific, Pittsburgh, USA) and 0.24 wt% solutions were used for fluorescent visualization of the patterning of the chips.

Equipment

Harvard Phd Ultra micropumps (Harvard Apparatus, USA) were used to dispense water and oil. A Zeiss Axio Imager Z1 optical microscope (Carl Zeiss, Germany) equipped with a Phantom micro 310 high speed camera (Vision Research Ltd., USA) and AxioCam CCD camera (Carl Zeiss,

Germany) was used to monitor the generation of double emulsion droplets. Contact angle measurement was performed using FTA 4000 (First Ten Ångstroms, USA).

The process used to fabricate the microfluidic devices is summarised in Figure 1. Microfluidic channels were defined by standard photolithography methods using a photoresist (SU8, microchem, USA) with channel sizes of depth 110 μm, and widths 85 μm, 150 μm and 250 μm for the first, second and third channel zones respectively. An additional chip with channel size of depth 23 μm, and widths 40 μm, 60 μm and 110 μm for the first, second and third channel zones respectively was also constructed to generate smaller double emulsion droplets (Supplementary S1). Soft lithography was employed to replicate the channel patterns, seal the microchannels and treat the PDMS surfaces at the desired locations. In detail, PDMS and a crosslinker were mixed in a 10:1 ratio (base: curing agent) and after curing at 60 ^{o}C for more than 2h, the PDMS slab was peeled off the mould. The inlets and outlet were punched using a 1 mm diameter biopsy punch (KAI industries, Japan). EVO-STIK epoxy (Bostik, UK) was then carefully patterned using a glass tip (visualised using an optical microscope) to block the channel zones targeted for hydrophobic surface properties (eg. zone 2, when preparing chips to generate W-O-W emulsions) on the channel layer and the corresponding area on a flat PDMS slab, demarcated by markers from the back side. The application of epoxy was performed using a glass capillary tip with diameter of 300 μm (Capillary Tube Supplies Ltd., Cornwall, UK). Both the channel layer and backing slab of the PDMS substrates were patterned with epoxy glue to ensure that all surfaces in Zone 2 were hydrophobic, such that they could support water in oil droplet generation. After the epoxy glue had solidified, the two PDMS slabs were treated using a plasma cleaner (Harrick Plasma, USA) for 2.5 mins. This time can be varied between 2 and 4 mins, but an exposure of at least 2 mins is recommended to render the PDMS slabs sufficiently hydrophilic; over 4 mins may result in weak bonding due to over oxidation. Finally, the microfluidic chip was prepared by quickly removing the epoxy using tweezers and then immediately aligning and bonding the PDMS slabs.

A camera image of a prepared double emulsion chip is shown in Figure 1. The entire chip is 2.5 cm in length and 1.5 cm wide and was constructed from PDMS alone; no additional chemicals were used to functionalise the surfaces such that this method is therefore very “clean”. It is also noted that the untreated area of PDMS is very small (≈ 1mm × 1mm) as compared to the large, plasma-treated area. It therefore has no effect on the bonding

between two PDMS slabs, and no leakage was observed at the site where the epoxy glue was employed.

Extending the Lifetime of the Double Emulsion Devices

Two approaches were investigated to extend the working lifetimes of our double emulsion chips. In the first, the channels of newly-prepared chips were immediately filled with DI water by capillary action, where this reduces the rate of hydrophobic recovery, as compared with storing the microfluidic devices in air. Our second approach investigated solvent-extraction as a means to extract uncured oligomers from cured PDMS and was shown to dramatically reduce the rate of hydrophobic recovery. PDMS extraction of both the patterned channel layer and the backing slab was performed immediately after curing using Soxhlet extraction (Supplementary S2) in 2-propanol,[26] This extraction process was conducted at 145 ^{o}C for 4 days and the PDMS was then placed in a fume hood overnight to facilitate evaporation of any residual 2-propanol. The "extracted" PDMS elements were then used to construct microfluidic devices as described previously.

Characterisation of the Wettability of the Microchannels

The successful spatial control of surface wettability was then tested, where the results are shown in Figure 2. Figure 2a shows a PDMS slab in which a microchannel is protected using epoxy glue in zone 2. The glue automatically fills zone 2 and then stops at its edge due to capillary forces. This is facilitated due to good wetting of the PDMS by the epoxy glue. The design of the devices also makes it very easy to specifically pattern one zone in the microchannels. Figure 2b in turn illustrates the hydrophilicity of zone 3 and the hydrophobicity of zone 2 in devices that were plasma treated for 2.5 mins and then bonded to form an enclosed channel.

Figures 2c and 2d show fluorescent images of zone 2 and zone 3 respectively after flushing a small drop of 0.24 wt% Rhodamine 6G solution through the channels. It is clearly seen that no water remains in Zone 2, while many water droplets reside in Zone 3. This provides good confirmation of the hydrophobicity of Zone 2 and the hydrophilicity of Zone 3 and shows that the epoxy glue successfully preserves the hydrophobicity of Zone 2. A success rate of over 95% was achieved for this patterning process, as assessed by testing over 20 PDMS slabs with channels (12 are shown in Supplementary S3). A successful rate of over 70% was achieved for the entire chip fabrication process (as determined for over 20 devices), where this includes the

mould replication, Soxhlet extraction, coating and alignment bonding procedures. Unsuccessful devices typically resulted from dust residues in the channels or misaligned bonding.

Results and Discussion

Having demonstrated that treating designated areas of the PDMS chip with epoxy glue provides an effective method for achieving patterning of the wettability, we then explored the use of this strategy to create devices that can generate W/O/W and O/W/O double emulsions. We also investigated the lifetimes of our devices, where the recovery of hydrophobicity in surface-treated PDMS is well-recognised to be a significant problem, and can rapidly render such PDMS devices useless. PDMS that had not been solvent-extracted ("conventional" PDMS) and PDMS that had undergone extraction ("extracted" PDMS) were employed.

Surface Wettability

Long term hydrophilic stability of PDMS devices is essential for the reliable generation of double emulsions over the time-periods required for practical applications. While oxidation of PDMS using plasma treatment makes the surface hydrophilic, typically this effect only lasts for several hours,[27] after which time the hydrophobicity recovers. This recovery effect is due to the migration of uncured low molecular weight oligomers from the bulk PDMS to the surface, and the reorientation of polar groups into the bulk.[28] The ability to create microfluidic devices with long-lived surface modifications is therefore extremely appealing, particularly when these can be used to create chips with both hydrophilic and hydrophobic channels.

In order to gain further information about the hydrophobic recovery of plasma-oxidised PDMS (both "conventional" and "extracted"), we explored the rate at which this occurs in different media. This was performed by recording the contact angles of water droplets placed on plasma-treated PDMS substrates that had been aged using a range of methods. As shown in Figure 3, non-oxidized, conventional PDMS exhibits a contact angle of 102.8^{o}, where this is reduced to <20^{o} immediately after plasma-treatment. Rapid recovery of hydrophobicity was observed on incubating the plasma-treated PDMS in air. A contact angle of 39.5^{o} was recorded after the PDMS had been left in air for 4h, and 83.1^{o} after 24 h. Similar hydrophobic recovery was observed when plasma-treated PDMS was placed in an oven at 60 ^{o}C for 24h (92.0^{o}) or immersed in water for 72h and then exposed to air for 24h (79.1^{o}). This demonstrates that hydrophobic recovery of plasma-treated PDMS will occur after 24 h in air, at room and

elevated temperatures. Recovery occurs more rapidly at higher temperatures, where this can be attributed to the faster diffusion rate of oligomers from the bulk PDMS to the surface. Importantly, a much reduced rate of recovery was observed when the PDMS was immersed in water for 72 h (33.9^{o}) rather than air (about 85.0^{o}).

Finally, the hydrophobic recover of "extracted" PDMS was also investigated. Contact angles of 57.0^{o} and 67.6^{o} were recorded for plasma-treated, extracted PDMS that had been incubated in air for 72 h or an oven at 60 ^{o}C for 72 h respectively. This compares with 83.1 ^{o} and 92.0^{o} for conventional PDMS after just 24 h of the same treatments. Solvent extraction of PDMS therefore significantly enhances the lifetime of the hydrophilic surfaces created by plasma treatment.

We speculate that the significantly slower rate of hydrophobic recovery for conventional PDMS immersed in water as compared with air can be attributed to a reduced rate of diffusion of oligomers to the surface and the suppression of reorientation of polar groups into the bulk. The surface energy of the PDMS in air will be reduced on increasing the surface hydrophobicity, while the converse will be the case for PDMS immersed in water. A slower rate of diffusion of the hydrophobic oligomers is thus expected for PDMS immersed in water, as is consistent with the study by Lawton et. al,29 who found recovery of PDMS from hydrophilic to hydrophobic is much slower in water and faster in hexadecane compared it in air. As the majority of oligomers were removed from the extracted PDMS its hydrophobic recovery is very slow and can be attributed only to the reorientation of polar groups at its surface. These simple experiments therefore clearly demonstrate that immersion of freshly-plasma treated PDMS in water and the use of extracted PDMS provides an effective route to maintain the hydrophilicity of plasma-treated PDMS.

Formation of W/O/W Double Emulsion Droplets

Figure 4a shows the formation of W/O/W droplets in a microfluidic chip prepared from conventional PDMS and immersed in water after bonding. The internal water droplets are formed at the first junction (which is hydrophobic) and are then carried downstream by the continuous oil phase. The water-in-oil stream is then sheared by the second water phase at the second junction, such that monodisperse double emulsion droplets are generated. Figure 4b shows the uniformity of the W/O/W droplets, where a flow rate of 30 µl h^{-1} – 50 µl h^{-1} – 500 µl h^{-1} (inner water – oil phase – outer water) generates 256 µm droplets with 167 µm cores

(Supplementary movie1). This microfluidic chip was re-evaluated for double emulsion production 3 days after its original fabrication and still worked well.

The ability to precisely control the volume encapsulated in the cores, and the number of cores per droplet, is significant for double emulsion microfluidic chips. This can readily be achieved in our devices by varying the flow rates of the inner (Q1), middle (Q2) and outer (Q3) fluid streams. Looking at the formation of W/O/W double emulsions, control over the sizes of the droplet cores was achieved by varying the flow rate of the inner water stream (Q1), while holding the flows of the middle oil phase (Q2) and outer water stream (Q3) constant (Figure 5a). Exemplifying with flow conditions in which the oil phase (Q2) and the outer water (Q3) were maintained at 50 µl h^{-1} and 500 µl h^{-1} respectively, these experiments showed that the core size increased with increasing flow rate Q1. This is clearly seen in the images of the double emulsion droplets in microchannels, where these are presented adjacent to their respective data points in Figure 5a. Further analysis of the data demonstrated a nearly linear relationship between the core size of the double emulsions (as imaged on-chip) and the flow rate. This relation was obtained by measuring and averaging over 10 sets of droplets. These experiments were also repeated using fluorocarbon oils (e.g. HFE-7500 or FC-40) in place of the sunflower oil, where these possess much lower viscosities, and with FC-40 containing 2.5% raindance surfactant.[30] Again, highly reliable production of double emulsions was achieved.

The fluid flows within the microchannels could also be varied to allow definition of the number of cores per double emulsion droplet. Multiple internal aqueous cores were generated by decreasing Q3, where the reduced flow rate of the outer water phase causes more water droplets to accumulate before they are sheared. Figure 5b shows an example of a double emulsion containing 2 core droplets of sizes 192 µm, where this was generated with a flow rate of 50 µl h^{-1} – 50 µl h^{-1} – 300 µl h^{-1} (inner water - oil phase – outer water).

We also investigated the formation of W/O/W double emulsions in devices constructed from extracted PDMS that had been left in air (rather than incubating in water) for 3 days prior to use. Figure 6 shows a snapshot of double emulsion generation in such a chip, where it is clearly seen that Zone 2 preserves hydrophobicity for water droplet generation and Zone 3 preserves hydrophilicity for oil droplet generation (Supplementary movie 2). This striking result clears the way for the production of stable microfluidic chips with patterned surface wettability.

Our method was also extended to generate PDMS devices capable of producing double emulsion droplets less than 100 μM in size, where this opens up possibilities including analysis in commercial fluorescence-activated cell sorting (FACS) machines, which have nozzle sizes of around 100 μM. Chips with similar designs but with smaller channels were prepared as described in the device fabrication section. The entire fabrication method is unchanged as compared with the larger devices. Fig. 7(a) shows double emulsion droplets with sizes of 72 μm (inner core 24μm) that were produced under flow rates of 30 μl h^{-1} – 50 μl h^{-1} – 500 μl h^{-1}(Q1 – Q2 – Q3). Even smaller droplets – 60μm (inner core 29μm) were formed on increasing Q3 to 700 μl h^{-1} and keeping Q1 and Q2 unchanged (Fig. 7(b)). To make the oil shell thinner, Q1 and Q3 were increased to 40 μl h^{-1} and 900 μl h^{-1} respectively, where this generated droplets with size of 55 μm (inner core 36μm). While the pressures required to form smaller droplets are significantly higher than those used to make their larger counterparts, no leakage of our devices was observed (over periods of ≈ 3 h). These results show the versatility of our method, where we have used it to generate double emulsion droplets with sizes from ≈ 50 μm to over 200 μm.

The reproducibility of double emulsion generation was also assessed in both the larger and smaller channel devices, where 1200 emulsion droplets generated from 5 of each chip type were analysed. A histogram displaying the size distribution of the droplets formed in these chips is shown in supplementary S4. The data demonstrate that ≈ 93% and ≈ 88% of the droplets are double emulsions in the larger and smaller channel devices respectively and that the droplets exhibit narrow size distributions. That double emulsion formation is somewhat less successful in the smaller as compared with the larger channel chips can be attributed to the higher pressure required to shear small droplets and an insufficient supply of water droplets from the first junction.

W/O/W and O/W/O double emulsions

O/W/O droplets, which can be considered to be the reverse of the previously described W/O/W double emulsions, are important in applications such as oil phase based chemical delivery and material synthesis. The ability to use the same chip design to create both W/O/W and O/W/O emulsions is clearly extremely attractive, providing practicality and convenience. Demonstrating the versatility of our fabrication process, reversed double emulsion (O/W/O) chips were also prepared using the same design principles. This was achieved using identical procedures to those employed to manufacture W/O/W chips, with the exception that zone 3

of the microchannel (rather than zone 2 as is required for W/O/W droplet production) (Figure 1) was now protected with epoxy glue such that it retains its hydrophobicity when exposed to the oxygen plasma.

The prepared chips were then used to generate O/W/O double emulsions, where superior visualization of these droplets was achieved by dyeing the water phase with blue food colouring. Figure 8a shows an image recorded at the second junction of O/W/O droplets generated with flow rates of 30 µl h^{-1} – 50 µl h^{-1} – 500 µl h^{-1} (inner oil – middle water – outer oil), where the blue dye clearly identifies the water phase between the two oil phases. For comparison, Figure 8b shows W/O/W droplets generated using the same flow rates of 30 µl h^{-1} – 50 µl h^{-1} – 500 µl h^{-1} (inner water – middle oil – outer water). These results clearly demonstrate how our simple experimental procedure can be used to prepare microfluidic chips that can generate W/O/W or O/W/O double emulsions, as desired.

Conclusions and Outlook

In summary, we have presented a reliable and perhaps most importantly – *simple* method – for preparing PDMS microfluidic devices for double emulsion generation. That it is also versatile is demonstrated by the fact that the same chip design can be used to create devices that generate either W/O/W or O/W/O double emulsions. Our approach relies on two key steps. Firstly, we achieve control over the spatial wettability of the microchannels by using epoxy glue to block the oxidation of specific regions of the channel layer and the backing slab during plasma treatment. The epoxy is then removed and the chip bonded immediately. This single step plasma treatment for creating selective wettability and achieving chip bonding eliminates cumbersome chemical surface-modification processes and enables microfluidic chips to be prepared in minutes. The second key element of our procedure is the maintenance of the patterned wettability. This was achieved either by filling the chips with water immediately after fabrication, or by constructing the devices from solvent-extracted PDMS and then storing them in air. Both procedures generated devices that reliably generated double emulsions for at least 3 days after manufacture. Indeed, it is expected that incubation of the extracted PDMS devices in water would yet further extend their active lifetimes. Importantly, this device manufacturing procedure can also be used to generate small double emulsion droplets (≈ 50 µm are demonstrated), which opens up further applications in materials science and biology. Our fabrication process therefore offers many advantages over existing methods for producing double emulsions using microfluidic devices, and we anticipate that this will

enable many more laboratories to produce and use double emulsions. It would also be entirely possible to extend our strategy to use a printer[31] to pattern the epoxy to achieve yet higher resolution and the mass production of multiple chips, if this was desired.

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Fig. 1 The fabrication process used to form the double emulsion microfluidic chips, where this includes soft lithography, epoxy glue patterning, plasma treatment and aligned bonding. The bottom right image shows a picture of the prepared microfluidic chip.

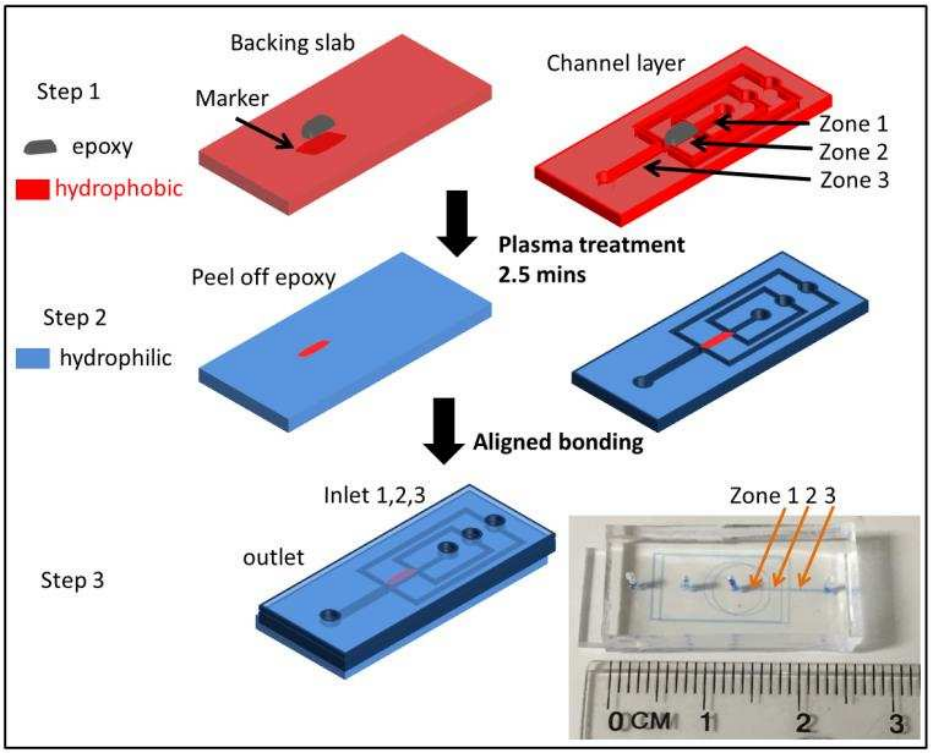

Fig. 2 Images of microchannels with spatial patterning of wettability. (a) Epoxy glue patterned at zone 2 before bonding, and (b) a device formed after plasma treatment and aligned bonding, showing hydrophilic (zones 1 and 3) and hydrophobic (zone 2) regions; (c) and (d) fluorescent images were taken in Zones 2 and 3 after flushing a small drop of 0.24 wt% Rhodamine 6G solution through the channels.

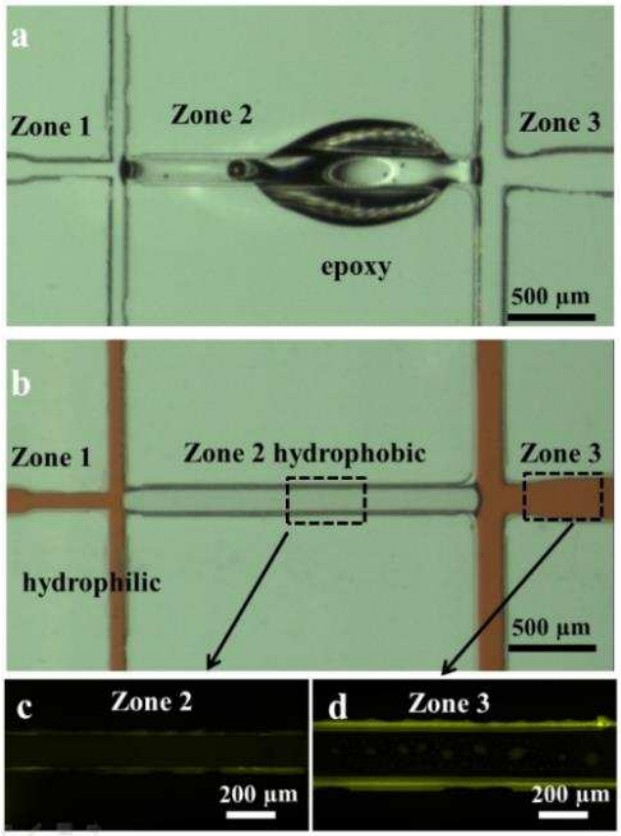

Fig. 3 Contact angle of water droplets placed on (a) untreated PDMS (102.8°), and PDMS that has been treated by (b) plasma treatment, followed by 4h in air (39.5°), (c) plasma treatment followed by 24h in air (83.1°), (d) plasma treatment followed by 24h in an oven at 60°C (92.0°), (e) plasma treatment then submerged in water for 72h (33.9°), (f) immersed in water for 72h and then exposed to air for 24h (79.1°), (g) PDMS after extracting uncured oligomer (98.1°), (h) plasma treatment of extracted PDMS, followed by 72h in air (57.0°) and (i) plasma treatment of extracted PDMS, followed by 72h in an oven at 60°C (67.6°). Freshly treated PDMS displays contact angles of < 20°.

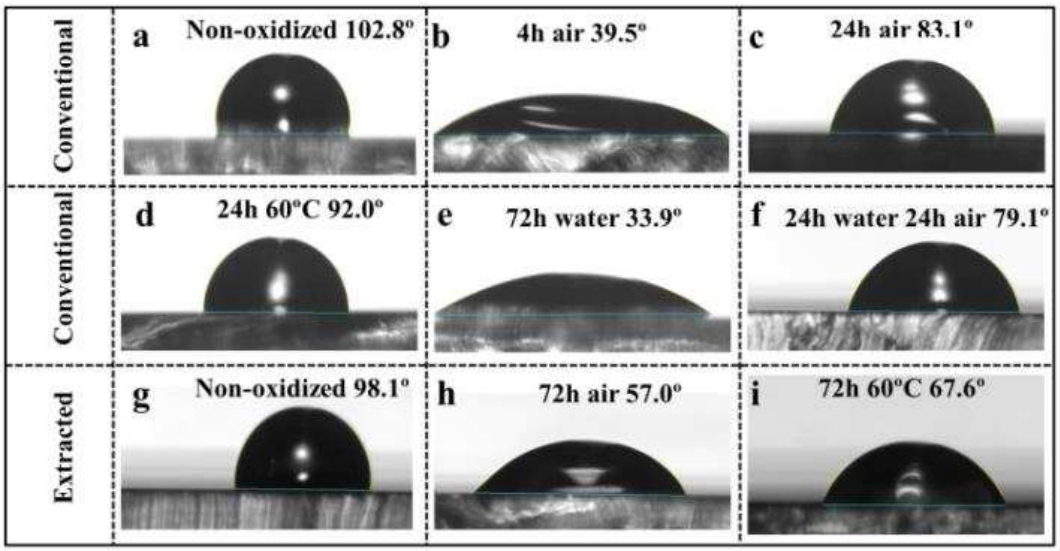

Fig. 4 Images of (a) the second junction forming W/O/W droplets, and (b) the collected W/O/W double emulsion droplets generated at a flow rate of 30 µl h^{-1} – 50 µl h^{-1} – 500 µl h^{-1} (inner water – oil phase – outer water).

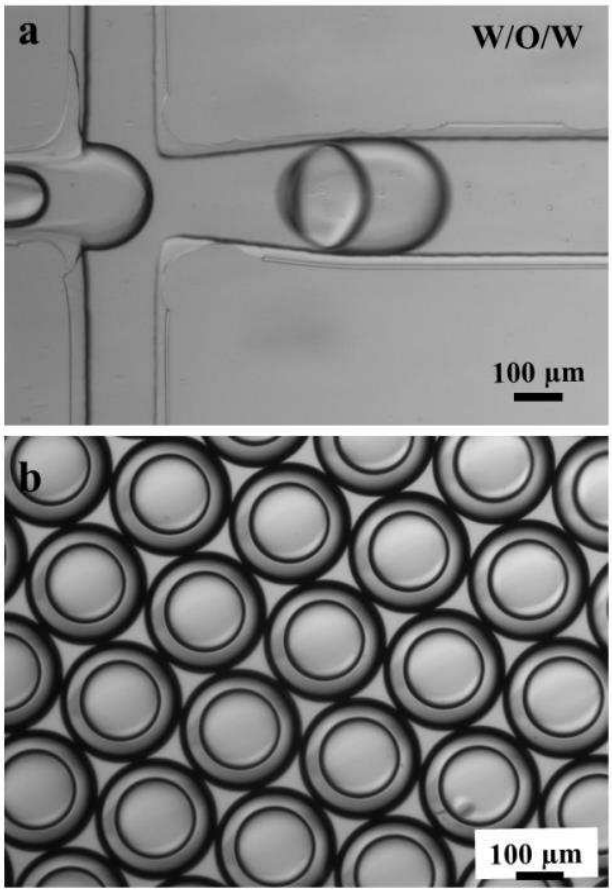

Fig. 5 Production of W/O/W double emulsions. (a) Graph showing the relationship between the core size and the Q1 flow rate, where this can be fitted to a linear relationship. The Q2 and Q3 flow rates are 50 µl h^{-1} and 500 µl h^{-1} respectively. The insets show optical micrographs of the double emulsion droplets formed at each of the associated data points. (b) 2 core double emulsion droplets with core sizes of 192 µm, where these are formed at flow rates of 50 µl h^{-1} – 50 µl h^{-1} – 300 µl h^{-1} (Q1 – Q2 – Q3).

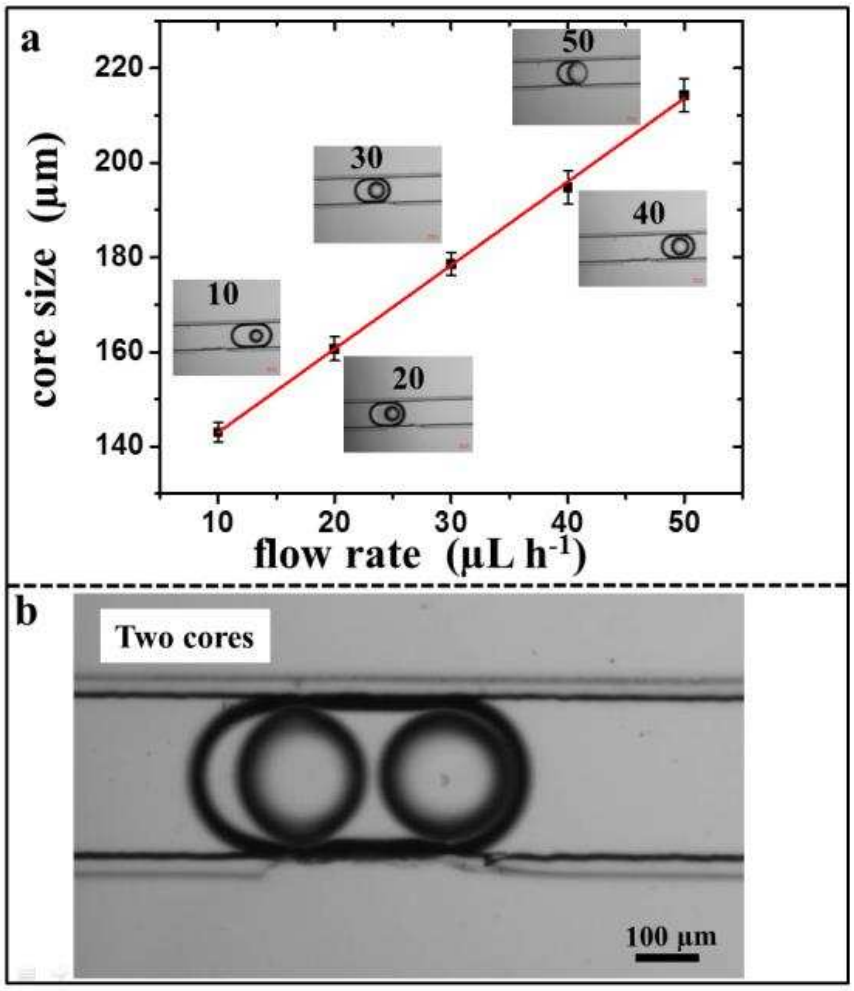

Fig. 6 Picture of W/O/W double emulsions generated by a chip prepared using extracted PDMS. The flow rates were kept at 30 µl h^{-1} – 50 µl h^{-1} – 500 µl h^{-1}(Q1 – Q2 – Q3).

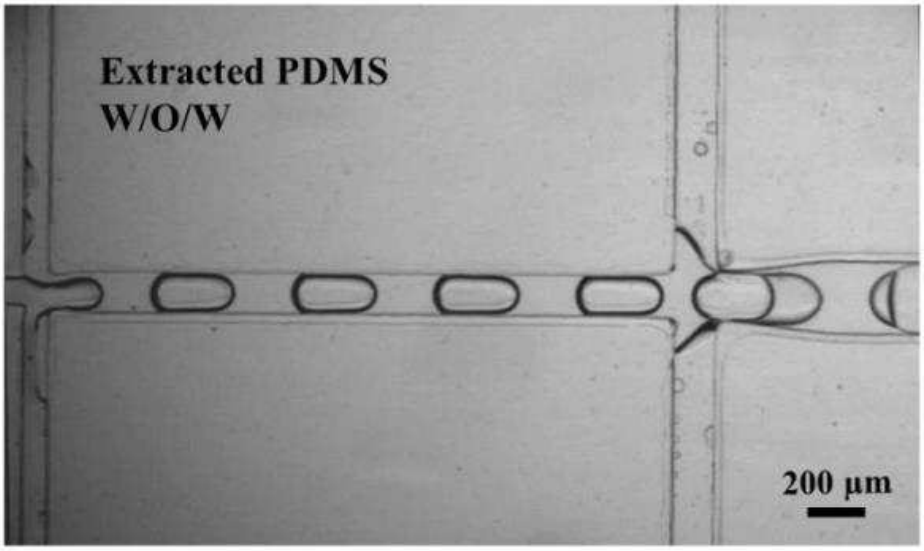

Fig. 7. W/O/W double emulsions generated by the chip shown in Fig S1: (a) 72 µm (inner core 24µm) droplets, (b) 60 µm (inner core 29µm) droplets, (c) 55 µm (inner core 36µm) droplets. These were produced under flow rates of 30 µl h^{-1} – 50 µl h^{-1} – 500 µl h^{-1}, 30 µl h^{-1} – 50 µl h^{-1} – 700 µl h^{-1} and 40 µl h^{-1} – 50 µl h^{-1} – 900 µl h^{-1} (Q1 – Q2 – Q3) respectively.

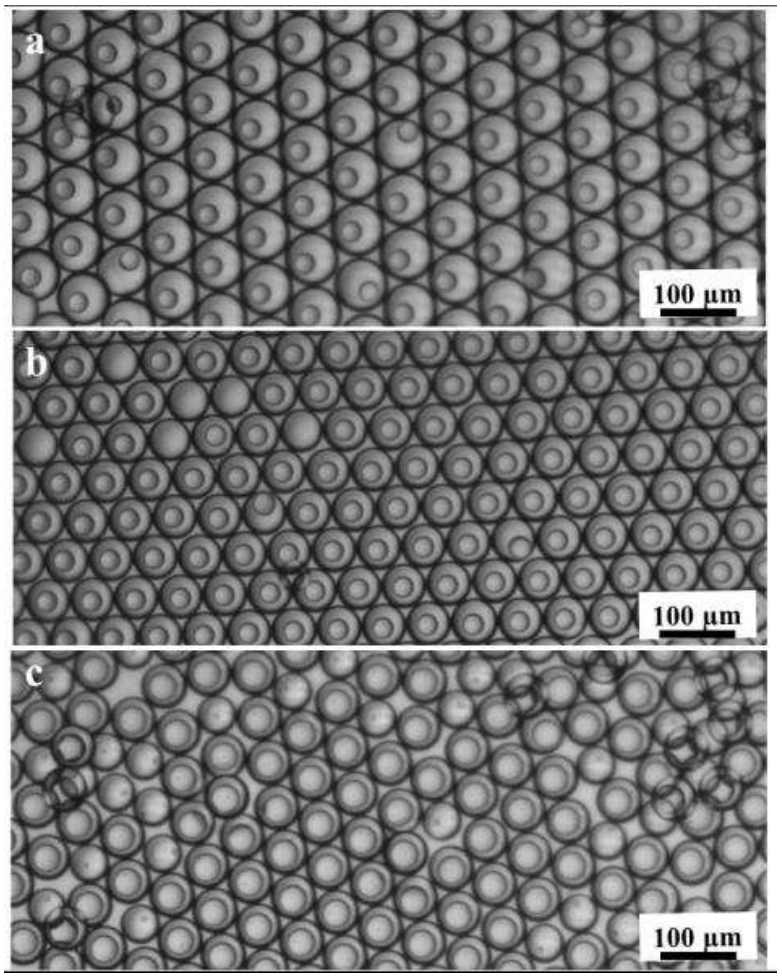

Fig. 8 (a) O/W/O droplets generated with flow rates of 30 µl h^{-1} - 50 µl h^{-1} – 500 µl h^{-1} (inner oil – middle water – outer oil). (b) The W/O/W microfluidic chip operating with a flow rate of 30 µl h^{-1} - 50 µl h^{-1} – 500 µl h^{-1} (inner water – middle oil – outer water).