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A 3D-Printed Microcapillary Assembly for Facile Double Emulsion Generation

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The design, fabrication and testing of facile microcapillary device assembly, suitable for monodisperse double emulsion production is reported. The interface is fabricated in a direct and rapid manner via 3D printing and shown to be robust in the controllable generation of both single and double emulsions at high generation frequencies.

Introduction

Double emulsions are complex systems of immiscible liquids arranged in a way that one or more droplets of one fluid (A, the inner phase) are formed inside a droplet of another, immiscible, fluid (B, the middle phase), which is itself encapsulated in an outer phase in which B is immiscible (A or C, the outer phase) (Figure 1a). Double emulsions have found a diversity of applications and uses in bioanalytical science, chemical synthesis, the generation of liposomes and polymersomes, cell mimetics, cosmetics, and in sustained release pharmaceutical formulations. The large-scale production of monodisperse double emulsions is commonly achieved using microfluidic methodologies that rely on the use of glass or polydimethylsiloxane (PDMS) structures. Although glass microfluidic devices are fabricated using standard photolithographic techniques, which incur high costs and require access to sophisticated instrumentation, they can be used with almost any type of solvent and operate at a range of temperatures and pressures. This contrasts with PDMS microdevices which, although cheap and easy to produce, are employable only for a severely limited range of working phases. Although recent studies have reported glass coated PDMS devices and hybrid glass capillary-PDMS devices that are able to withstand a variety of organic solvents, the need for microfabrication and photolithography facilities, and the incompatible physical properties of disparate substrates still limits their use. Indeed, all-glass devices for double emulsion generation must be either purchased from a small number of microfluidic device manufacturing companies or fabricated in a laboratory via laborious glass etching techniques or glass capillary assembly. The second method is by far the most used and the most adaptable in the production of multiple compartments of different solutions, a goal difficult to obtain with the other approaches. In the current paper we report a novel glass capillary double emulsion system that avoids the drawbacks that manual glass capillary device assembly usually carries. Introduced for the first time in 2005 by Utada et al., the glass microcapillary device has been widely used for the generation of monodisperse double emulsions in fields as diverse as materials science, biology and biomedical engineering. This system allows the formation of a double emulsion in a single emulsification step via the flow-induced breakup of a tri-phasic coaxial flow. The inner and middle phases flow in coaxial glass capillaries and are brought into counter-flow contact with the outer phase. This contact causes the flow to break up, forming a double emulsion. The double emulsion thus formed flows through the collection capillary, together with the outer phase, reaching the outlet of the device (Figure 1b-c).

Despite its widespread adoption, the assembly of such microcapillary devices is laborious, operator-dependent and irreproducible. The standard fabrication method consists of inner capillary drawing and chemical surface treatment, coaxial capillary alignment, adjustment of the distance between the opposing inner capillaries and fluidic inlet interface connection. Within this fabrication process several challenges are well recognized. First and foremost, the delicate alignment of the inner capillaries as well as their separation are difficult to control and even more difficult to reproduce. Second, the adhesive required for sealing inlets and outlet often blocks the glass capillaries and compromises device function. Finally the assembled...
device cannot readily be disassembled, cleaned and chemically retreated for reuse.

Herein, we present a novel approach for microcapillary interface fabrication that relies on lithography-free rapid prototyping and overcomes the aforementioned fabrication problems without altering the working principle of the microcapillary device. 3D printing represents an ideal fabrication technique for interface construction since it allows direct generation of complex, three-dimensional structures that are otherwise only achievable using multiple processing steps, and at significantly higher costs. Such rapid prototyping methods have proved their utility in a number of microfluidic applications.44-47 Our approach relies on the fabrication of two connectors that hold the inner and outer capillaries in position and provide access to the contained fluids. The connectors are mounted in a specular fashion on a guide that can easily be mounted on a microscope stage. Each connector is made of two parts: a screw and a nut. The first part houses the inner capillary and is screwed onto the second part that holds the outer capillary. Significantly, the outer capillary can either be circular or square in cross-section. The screw mechanism allows a translational movement of the inner capillary on both sides that permits precise definition of the distance between the inner capillaries even while liquids are flowing (Figure 2).

**Capillary Device Fabrication and Assembly**

Glass inner capillaries (ID 0.70, OD 0.86 mm) (VitroCom, USA) were tapered using a micropipette puller (P-1000, Sutter Instruments, USA) and a microforge (MF-900, Narishige Group, Japan), whereas glass capillaries with circular (ID 1.50 mm, OD 1.80 mm) or square (ID 1.00 mm, OD 1.40 mm) cross-section were used as the outer channel. Glass surface treatment was achieved by soaking capillaries overnight in a 2% solution of n-octadecyltrimethoxysilane (376212, Sigma Aldrich, Switzerland) in isopropanol, to generate a hydrophobic or hydrophilic coating respectively. Treated capillaries were then dried with nitrogen gas. The cleaned parts were then assembled and connected to syringe pumps using PTFE tubing (ID 0.79 mm, OD 1.58 mm, Cole Parmer Instrument Company, USA).

**Device Testing and Operation**

The device was tested using deionized water as inner and outer fluids and Mineral oil (Mg516, Sigma Aldrich, Switzerland) with 2 wt % of SPAN 80 (S670, Sigma Aldrich, Switzerland) as the middle phase. Fluids were loaded into glass syringes (1000 Hamilton gastight syringes, Switzerland) interfaced with the capillary device and mounted on syringe pumps (Aladdin Al-1000, World Precision Instruments, USA). An area scan color camera (CR-GC06-H640x, Teledyne Dalsa, Switzerland), mounted on a microscope (Axiovert 100, Zeiss, Germany), and a 2.5x objective (Eiplan-NEOFLUAR, 442310, Zeiss, Germany) were used to image and monitor droplet formation.

Sealing of the assembly was assessed by observation of any leakage when liquids were pumped into the device. Holes with circular profiles, in both the nuts and screws, showed complete integrity when operated at flow rates ranging from 1 to 110 μl min⁻¹. Occasionally, liquid leakage was observed in nuts with square profiles due to the fact that the square capillaries did not have sharp edges. In such circumstances, the polymer/glass interface could be sealed using a few drops of acrylic glue (Acrylic 116, Evonik Industries, Germany), which could be easily removed when the device was disassembled and soaked in acetone. Figure 3 shows the final 3D printed assembly whereas Figure 1S (ESI) shows inner capillaries in both a square and a round outer capillary. Performance of the screw movement was assessed by observing and measuring the travel distance of the inner capillary in response to fine screw rotation. Using this method, the smallest distance that the capillary could travel within the assembly after fine manual rotation of the screw was calculated to be 2.4 μm. A video of the manually operated intercapillary distance adjustment is provided in the ESI and clearly demonstrates the maintenance of coaxial alignment.

In addition, to assess the reproducibility of capillary alignment within the assembly, the offsets of capillary centers both in our assembly and in the commonly used epoxy-based assembly were measured. This analysis indicated that epoxy fittings can have an offset of up to 42% in normal construction whereas the 3D-printed assembly showed
a maximal offset error of 10%. Details of the measurements are provided in the Figure 2S (ESI).

Device operation was characterized using a square glass capillary (OD 1.4 mm – ID 1.0 mm) and two round glass capillaries (OD 0.87 mm – ID 0.70 mm) with tapered tip diameters of 24 and 185 µm. The coaxial relationship of the inner capillaries was verified from microscopic images and the distance between capillaries adjusted manually by rotation of the screws and set to three discrete distances as shown in Figure 4a.

For these three configurations, the middle and outer phase flow rates were kept constant at 10 and 110 µl min⁻¹ respectively, whereas the inner phase flow rate was varied between 1 and 10 µl min⁻¹. It was observed that stable double emulsion generation occurred only for a few combinations of input flow rates. Configuration I produced stable double emulsions with only 1 inner droplet when the inner flow rate was set at 3 µl min⁻¹ (Figure 4b); configuration II generated double emulsions with both 1 and 2 encapsulated droplets when the inner flow rate was set respectively at 3 and 9 µl min⁻¹ (Figure 4c-d) and configuration III generated only stable double emulsions containing 3 droplets for an inner flow equal to 8 µl min⁻¹ (Figure 4e). Videos of stable droplet generation were analysed to assess the inner droplet dispersity (provided in the ESI). Moreover it was observed that single encapsulation achieved in configuration I was 20 ms faster than that obtained with configuration II but produced inner droplets with a larger coefficient of variation (Table S1). For other flow rates values herein mentioned, a combination of double emulsion together with oil droplet generation and/or water phase merging occurred (Figure 4f and 4g).

The complete flow rate dependence of these formation domains, for each configuration, is graphically summarized in Figure 3S.

Finally, the reusability of our device was assessed by producing W/O/W emulsions, then disassembling the device, chemically retreating the component capillaries, reassembling the device and then producing a O/W/O emulsion. Figure 5 shows the double emulsions obtained before and after the disassembly and chemical retreatment.

Conclusions

We have described a novel and simple approach for the assembly of a microcapillary device used for double emulsion generation. The interface provides for facile coaxial alignment of inner capillaries and variation of their reciprocal distance through rotation of the screws to which the capillaries are secured. Importantly, the advantages introduced by the proposed approach extend to device reusability, reduced waste and setup time. Moreover, the assembly strategy presented herein can be achieved through the use of milling techniques and the adoption of more resistant materials such as PEEK (shown in Figure 4S in ESI), aluminium or steel. Furthermore, the screw mechanism is well suited to interconnection with motors able to finely tune the distance between the inner capillaries, a desirable feature for industrial assembly. Finally, the use of just one connector housing the inner and the outer capillary can be employed for the formation of single emulsions, or as a droplet dispenser, which can also be converted into a micropipette system when the flow of the inner capillary is reversed. The droplet dispenser represents a cost-effective solution for single emulsion generation when photolithographic methods are inaccessible or solvents not compatible with PDMS are in use.
configurations, which were tested for double emulsion generation. For each configuration, middle and outer phase flow rates were maintained at 10 and 110 µl min⁻¹, respectively, whereas the inner fluid flow rate was varied from 1 to 10 µl min⁻¹. Stable double emulsions were produced at particular flow rate sets.

(b) Configuration I: stable double emulsion generation, with single droplet encapsulation, occurring when the inner flow rate was set at 3 µl min⁻¹; (c-d) Configuration II: stable double emulsion generation, with single and double encapsulation, occurring when the inner flow rate was set respectively at 3 and 9 µl min⁻¹. (e) Configuration III: stable double emulsion generation, with triple droplet encapsulation, occurring when the inner flow rate was set at 8 µl min⁻¹.

(f-g) Oil droplet generation and water phases merging episodes. Scale bar equal to 500 µm.

Fig. 5 Device reusability. (a) W/O/W emulsion generation achieved by localized treatment of the inner, outer and collection capillary. The intercapillary distance was set at approximately 330 µm whereas the inner, middle and outer phase flow rates were set respectively at 8, 30 and 200 µl min⁻¹. (b) O/W/O emulsion generation after device disassembly and chemical treatment of the capillaries. The device was reassembled and the intercapillary distance was set at approximately 370 µm. Flow rates of inner, middle and outer phase were set at 50, 50 and 7 µl min⁻¹. Scale bar equal to 500 µm.

Acknowledgements

This project was partially supported by the ETH Zurich Postdoctoral Fellowship Program and Marie Curie Actions for People COFUND Program and by the Swiss National Science Foundation Grant CR2312-146328. Authors are also grateful to Professor Andre Studart and Mr Philipp Wei Chen for the use of micropipette puller and microforge.

Notes and references