Ready... Set, Flow: Simple fabrication of microdroplet generators and their use in the synthesis of PolyHIPE microspheres

Florian Lapierre[1,2,3] **& Neil R. Cameron**[4] **& Yonggang Zhu**[2,3]

[1] Micro/Nanophysics Research Laboratory, RMIT University, Melbourne, VIC 3000
[2] CSIRO Manufacturing Flagship, Private Bag 33, Clayton, VIC 3168, Australia
[3] Melbourne Centre for Nanofabrication, 151 Wellington Road, Clayton, VIC 3168, Australia
[4] Department of Chemistry & Biophysical Sciences Institute, Durham University, South Road, Durham DH1 3LE, UK

E-mail: `florian.lapierre@rmit.edu.au`

Abstract. During the last decade, research and development in microfluidic devices have grown significantly due to their wide variety of applications in physics, chemistry and biology. A specific category of microfluidic devices focuses on the generation of micro-emulsions that can be used, for example, as chemical and biological reactors to synthesize new materials and perform assays. However, the traditional fabrication process of such microfluidic devices involves the use of special instrumentations and clean room facilities. These steps are generally expensive, time consuming and require specific levels of expertise. In this article, an alternative method to fabricate robust microfluidic devices using conventional components and a new commercially available self-setting rubber is presented. Using this method, a cheap, reproducible and easy to manufacture microfluidic device has been developed for generating single and double emulsions in the range of 100s of μm with high throughput. One possible application of this device is demonstrated with the synthesis of highly porous polymer beads from a High Internal Phase Emulsion (HIPE). The fabricated ~~lunar-like~~ microbeads could find potential application in 3-dimensional cell culture due to their high porosity (up to 95 %) and pore size (from 5 to 30 μm). The process here presented offers great opportunities for chemists and biologists interested in synthesising new materials while minimizing time, costs and expertise in the development of microfluidic systems.

1. Introduction

Over the last 20 years, the interest in microfluidic devices has grown significantly due to their wide variety of multidisciplinary applications.[1] Microfluidic devices are widely used in many modern experimental and industrial workflows for manipulating, processing and controlling fluids at small scales (from micro- to picolitres).[2, 3] By using small fluid volumes, microfluidic devices can be used to:

- Scale up experimental work to thousands of runs using the same device, while simultaneously minimising the quantity (and therefore cost) of required fluid reactants;
- Minimize the amount of wasted material in industrial and experimental processes and improve recovery of reactants and products for reuse; and
- Maintain strict control over the synthesis of novel materials.

One application of these microfluidic devices is the synthesis of various types of emulsions for cosmetic, food, medicine and biotechnological industries.[4] Microfluidically generated emulsions have the advantages of monodispersity, controlled shape and internal structure, and surface properties.[5] These emulsions are currently used as chemical and biological reactors to synthesize new materials.[6, 7, 8] There has been an increasing demand for devices capable of generating emulsion droplets enclosed in an oil or aqueous environment, with size ranging from a few to 100s of μm in diameter. However, despite much progress in the development of microfluidic technologies, microdevices generally have to be fabricated through micro- and nano-fabrication technologies and/or operated in a clean laboratory environment. This not only increases considerably the cost of each microsystem and the time of fabrication but also limits or even prohibits the microfluidic development for large-scale industrial applications. There is a great need for simple and easy- to-make fluidic devices that can overcome some of these issues. The aim of this research is to develop a method for rapidly fabricating fluidic device for microdroplet generation without using cleanroom facilities. Microfluidic chips for microdroplet synthesis are normally fabricated in PDMS or hard polymeric materials such as PMMA, PC and COC.[9, 10] However, scientists also developed alternative methods to fabricate droplet generation devices. Weitz *et al.* introduced novel capillary-based microfluidic devices to generate single and liquid-monomer multiple-core droplets. By adjusting the surface chemistry on the capillary wall, single, double, and triple emulsions were produced.[11, 12] However, the fabrication of such devices is extremely challenging. The devices are assembled with needles and glass capillaries bonded together with epoxy on a glass slide. Reproducibility in fabrication is much more difficult than using conventional PDMS technology. Moreover, cleaning steps are limited by the presence of epoxy and the impossibility to disassemble the device.[13] In more recent papers, alternative methods[14, 15, 16] were presented by modifying a microfluidic device based on co-axial capillaries.[17] The new capillary system enables the preparation of water-in-oil (W/O) and oil-in-water (O/W) micro emulsions as well as easy disassembly of the device for cleaning and reuse. ~~However, although the unit is assembled conveniently, their method does not allow to monitor the droplet formation region.~~ In the same principle of simplification, we present in this article the fabrication process of an alternative microfluidic device for the synthesis of monodisperse emulsions based on the use of a new self-setting silicon rubber: Sugru®. The emulsions are generated through the same flow focusing process of the standard droplet-based microfluidic devices, but this system is fabricated with conventional and inexpensive components

only. It requires less than 10 minutes to fabricate the device and generate the droplets, and can be disposed of or recycled after use. In this paper, the fabrication method of this microdroplet-based microfluidic device is described; the functionality of the device is demonstrated through the generation of water-in-oil emulsions with controllable size. Moreover, an interesting application of this device is presented. The microdroplet generator was used to easily and quickly synthesise highly porous polymer microparticles that can be used for tissue engineering, 3-dimensional cell culture, biocatalysis and hydrogen storage materials.

2. Materials and Methods

2.1. Materials

All reagents were purchased from Sigma/Aldrich (Australia) unless otherwise indicated. Polypropylene pipette tips (200 μL) were purchased from LabAdvantage. Transparent silicon tubing was bought from Geko Optical and transparent PTFE tubing from Cole-Parmer. Silicon tubing (Inner diameter (ID): 0.5 mm, Outer Diameter (OD): 1.3 mm) has the advantage of flexibility with excellent memory characteristics. PTFE tubing (ID: 0.3 mm, OD: 0.76 mm) is more rigid and can be used in harsh chemical environments. The self setting rubber was provided by Sugru® (FormFormForm Ltd.). Sugru® is a patented multi-purpose and malleable silicon rubber. Exposing this material to air causes the rubber to self react and form a cured polymer. Sugru® can adhere to a large range of surfaces such as aluminium, glass, copper, ceramics, etc. but does not bond to polypropylene. Four syringe pumps were purchased from Cetoni (neMESYS pumps).

2.2. Pipette tip based microchip fabrication

The fabrication process of droplet-based microfluidic devices with conventional components is presented. These chips are fabricated at low cost (few cents per chip), in a short period of time (< 10 min before droplet generation) without any clean-room facility, microscopes or epoxy glue requirements. The device is based on a standard flow focusing technique[18, 19] and is fabricated as follows. First, the mould of the inner part of a micropipette tip was fabricated with the Sugru® rubber including two PTFE tubes, placed inside the micropipette (Fig. 1.a). These pieces of tubing will create spaces in the mould for the 2 future inlets. The rubber was polymerized for 24 hours before pipette tip and tubing removal (Fig. 1.b). The cured mould can be inserted in any analogous pipette tip and reused indefinitely. The mould resists solvents as well as basic chemicals used for cleaning. The use of a polypropylene tip prevents the rubber from binding while moulding the inner shape of the pipette. Once the Sugru® rubber had cured and the tip and tubing were removed, two new PTFE tubes were inserted into the designed spaces of the mould. The elasticity of the rubber ensures robust sealing. These two tubes were connected to two syringe pumps, for the delivery of the continuous and dispersed phases respectively (Fig. 1.c). A 2 mm long PTFE tube was placed inside

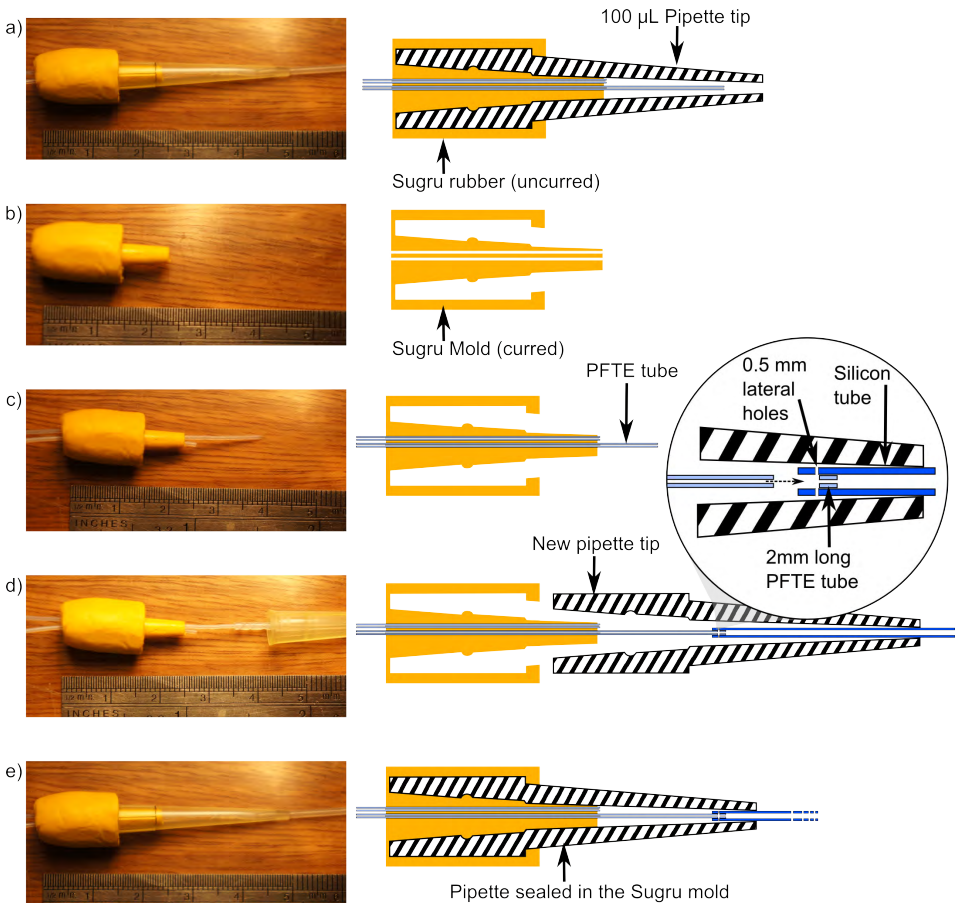

Figure 1. Schematics and pictures of the low cost droplet-based microfluidic device. (a) Fabrication of the silicon rubber mould inside a polypropylene micropipette tip. (b) Insertion of two rigid PTFE tubes inside the silicon rubber mould. (c) Insertion of one 2 mm long PTFE tube inside a flexible silicon tube. This silicon tube was inserted in the micropipette tip. The junction between the PTFE and silicon tubes was punched to obtain the nozzle part of the flow focusing device. (d) Insertion and sealing of the micropipette tip containing the tubing with the silicon rubber. After creating the rubber mould (reusable), this device can be ready in less than 10 min using low cost, off-the-shelf components.

a silicon tube (tube length depends on users needs) at 1 cm distance from one of the extremities of the silicon tube. The silicon tube inner diameter and the PTFE tube outer diameter ensure a robust sealing. A 0.5 mm punch was created on the lateral wall of the silicon tube and next to the PTFE tube using a biopuncher from Ted Pella, Inc (Harris Uni-Core). These steps determined the creation of the nozzle part of the flow focusing microfluidic device. It is to be noted that the 2 mm long PTFE tube can be replaced by any other tubing or glass capillary to obtain various sizes at the nozzle part. The silicon tube was then inserted in a new pipette tip while the PTFE tube delivering the dispersed phase was placed in the silicon tube, next to the nozzle (Fig. 1.d). Again, the flexibility of the silicon tube allows a complete sealing of both the pipette tip and the PTFE tube. The other PTFE tube will fill the pipette tip with the continuous phase.

Finally, the pipette tip was inserted in the Sugru® mould to seal the device (Fig. 1.e). For water-in-oil (W/O) emulsion, the tube delivering the water (dispersed phase) was connected to the silicon tube. For oil-in-water (O/W) emulsion, the 2 mm long PTFE nozzle was replaced by a hydrophilic glass capillary with the same dimension and the PTFE tube delivering oil (dispersed phase) was connected to the silicon tube. It is to be noted that the hydrophilic glass capillary can be longer than 2mm to avoid the oil droplets binding to the wall of the silicon tube. This case is only valid when oil droplets are as large as the tubing diameter (results not shown).

2.3. Dispersed and continuous phases preparation

The functionality of the microfluidic device was verified by generating water-in-oil (W/O) and double water-in-oil-in-water (W/O/W) emulsions of various dimensions. The aqueous phase was prepared by mixing deionised water with red or blue food dye (for imaging); the oil phase was fluorinated oil Novec 7500 containing 2 % Picosurf surfactant. To generate W/O emulsion, the oil (continuous phase) filled the pipette tip while the aqueous solution (dispersed phase) was delivered in the silicon tube towards the nozzle outlet. For double W/O/W emulsions, the outer aqueous phase was composed of deionised water with 2 % Tween 20 surfactant. A second microfluidic chip was connected to the outlet of the first chip, thus creating a train of two devices. A hydrophilic glass capillary replaced the hydrophobic PTFE tube at the nozzle part of the second device. The continuous aqueous phase filled the pipette tip while the first emulsion was delivered through the dispersed phase's tubing towards the second nozzle outlet.

2.4. Micro-emulsions generation through the pipette tip-based microchip

The microfluidic device was used to generate W/O emulsions. While the continuous and dispersed phases were delivered through the syringe pumps into the device, air was expelled from the nozzle part by priming it with oil. Then, oil and water flow rates were adjusted at 2:1 ratio at 100s μL/h, to generate the W/O emulsion at the nozzle part. The flow rate of each phase as well as the diameter of the nozzle determined the droplet size as defined by the flow focusing technique. For instance, by using 100 μm ID PTFE tubing (Fig 2.a), it was possible to generate 100 μm droplet emulsion (Fig 3.a) with high monodispersity (Fig 3.b). The same principle was applied with a 300 μm ID PTFE tube to generate a 300 μm droplet emulsion with similar flow rate (Fig 2.b). Without any nozzle (Fig 2.c), the size of the droplets was mainly dependent on the silicon tube inner diameter as well as the oil and water flow rates. In this case, 500 μm droplet emulsion were obtained. Therefore, by selecting the size of the tubing at the nozzle part, it is possible to modulate the diameter of the emulsion droplets from one hundred microns to sub-millimetres. It is to be noted that the device can handle overall flow rates up to 10 mL/h with these fluids without any leakage. Double W/O/W emulsions were also generated by using a train of microfluidic devices. By adjusting the flow rate of the three phases, it was possible to generate double emulsions containing

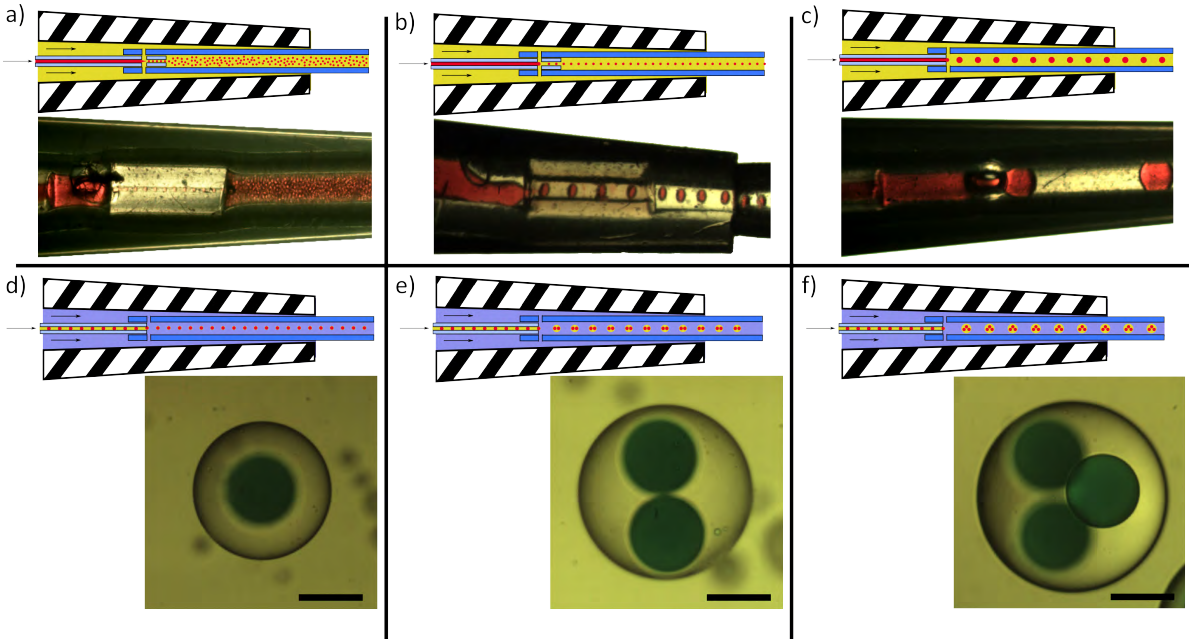

Figure 2. Schematics and pictures of the flow focusing junction where the single emulsions are generated with a (a) 100 μm, (b) 300 μm, or (c) 500 μm nozzle PTFE tube. Schematic and pictures of the flow focusing junction where double water in oil in water emulsions are generated with (d) one (e) two, or (f) three aqueous droplets with optical micrographs of monodisperse double emulsions. In the schematics, the inner aqueous phase is represented in red, the oil phase, in yellow and the outer aqueous phase in blue. Black scale bar is 100 μm.

one (Fig 2.d), two (Fig 2.e) or three (Fig 2.f) aqueous droplets with 100 μm diameter.

2.5. PolyHIPE preparation

In order to operate the pipette tip based microfluidic device in an applicative domain, highly porous polymer (polyHIPE) beads from a High Internal Phase Emulsion (HIPE) were synthesized. The HIPE formulation was prepared as detailed by Kircher *et al.*[20] and by Kimmins *et al.*[21] and injected as an aqueous phase in the microfluidic platform. Briefly, the monomers 2-ethylhexyl acrylate (5.0 g), isobornyl acrylate (4.0 g), and dipentaerythritol penta/hexa-acrylate (1.0 g) were mixed in a round bottomed flask. Hypermer B246 surfactant (0.3 g) was added to the mixture and stirred mechanically with a paddle stirrer until dissolution. Then, 0.1 g of diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide/2-hydroxy-2-methyl-propiophenone blend photoinitiator was added in the solution. The resulting mixture was stirred at 300 rpm while deionized water (70mL) was added dropwise. HIPE at 80 vol % water content was thus obtained.

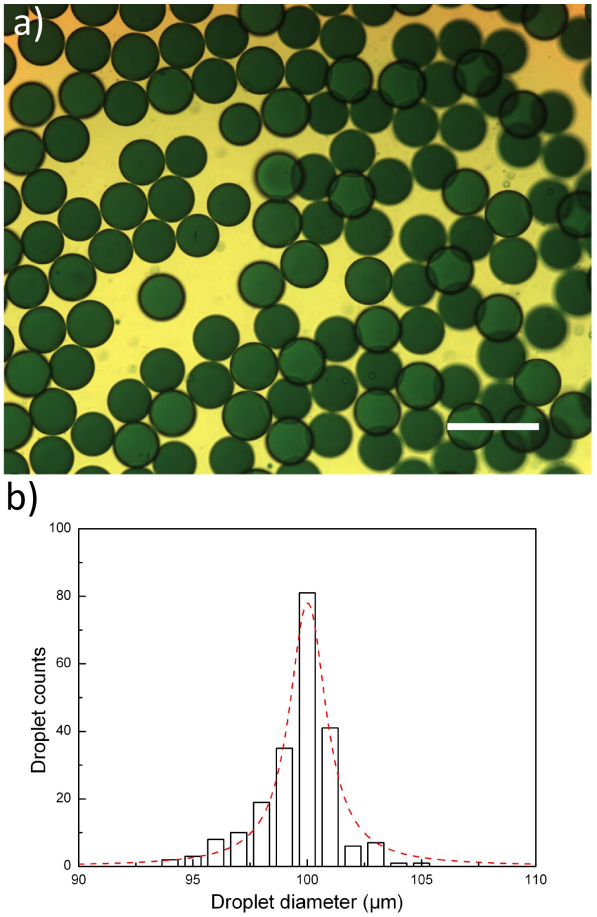

Figure 3. (a) Magnified picture of blue food dye aqueous droplets in Novec 7500 generated with the pipette tip-based microchip and a 100 μm ID PTFE tube at its nozzle. (b) Histogram showing the monodispersity of the droplets at 100 μm. The image processing of the droplet led to 1.74 μm standard deviation. Scale bar is 200 μm.

2.6. Fabrication of monodisperse porous polymer beads

An interesting application of the microfluidic device consists of the generation of monodisperse porous polymers beads. In this case polyHIPE emulsions were prepared and used as dispersed phase, while fluorinated oil Novec 7500 containing 2 % Picosurf surfactant constituted the continuous phase. HIPE droplets were polymerised by UV polymerisation while still inside the silicon tube (see video in ESI) and collected in a plastic vial (Fig. 4.a). Beads were then washed with acetone and dried in a vacuum oven at 50 °C for a minimum of 24 hours. 200 μm diameter polyHIPE beads were generated with high monodispersity and 80 % nominal porosity (Fig. 4.b-e). Internal pores size varied from 5 to 30 μm with very high polydispersity. This is explained by the generation of the first W/O emulsion by stirring while producing the PolyHIPE precursor emulsion. These PolyHIPE bead materials could be used for a wide range of applications, including tissue engineering, 3-dimensional cell culture, biocatalysis and

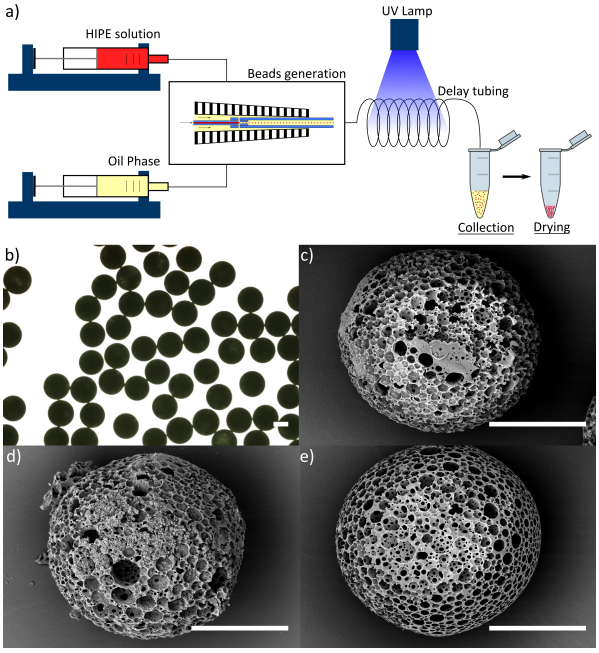

Figure 4. (a) Schematic of the HIPE microparticles using pipette tip based microfluidic system and UV lamp to generate the particles and activate the polymerisation prior collection. HIPE is represented in red while the oil phase in yellow. After 24 hours drying, beads were analysed under SEM. (b) Magnified picture of the monodisperse HIPE beads. (c-e) SEM pictures of 3 HIPE microparticles. White scale bar is 100 μm.

hydrogen storage materials. This is one example among many potential applications that this device can offer for biologists and chemists in order to generate microparticles and develop new materials.

3. Discussion and Conclusion

An innovative method for fabricating off-the-shelf microfluidic devices for controllable production of monodisperse emulsions has been developed. This method does not involve any specialized equipment or facilities such as photolithography, or dirty and hard to use epoxy or silicon paste. By combining a micropipette tip with tubing in the appropriate materials and size, the microfluidic device was fabricated within 10 minutes (post rubber moulding) without requiring any microfabrication expertise. The Sugru® rubber is the key component of the system as it ensures total sealing and can be "indefinitely" (So far, we have recycled the same silicon rubber for each experiment). The Sugru® rubber is relatively cheap (around £11/40 g), self-setting, hand-formable, self-adhesive and flexible when cured. The pipette tip based microfluidic device was proved to generate monodisperse emulsions with diameters ranging from 100 to 500 μm with the adequate tubing. A larger range of droplet diameters could be generated by replacing the nozzle part with a microinjection needle processed with a capillary puller; double emulsions can also be easily obtained through a train of

microdevices. The combination of hard PTFE or flexible silicon tubes within the device give users modularity in adapting the microfluidic device for particular applications; hard tubing will ensure robustness, pressure stability (no tube deformation) prior droplet generation while soft and flexible tubes will provide adequate sealing around the micropipette tip. It is to be noted that both tubes are hydrophobic and are therefore suitable for W/O emulsion. In the case of O/W emulsion, appropriate hydrophilic glass capillary was inserted inside the flexible silicon tube. The sealing around the pipette tip is still maintained while providing hydrophilic coating for such emulsion. An example application of the off-the-shelf microfluidic device is the synthesis of monodisperse polyHIPE porous microparticles that can be generated with high throughput in controllable sizes. In conclusion, the new fabrication method we presented in this paper allows quick and cheap development of an easy to use and reusable platform for monodisperse emulsion droplet generation. This platform can be used in a multidisciplinary range of applications without requiring any expertise in microfabrication and fluid dynamics. This new concept will offer chemists and biologists an unprecedented tool for designing and synthesising new materials.

Acknowledgments

This work was performed in part at the Melbourne Centre for Nanofabrication (MCN) in the Victorian Node of the Australian National Fabrication Facility (ANFF). The authors would like to thank Sugru® (FormFormForm Ltd.) for providing samples in order to fabricate and assemble the devices. NRC is the recipient of a CSIRO OCE Distinguished Visiting Scientist award.

References

[1] Whitesides G M 2006 *Nature* **442** 368–373 ISSN 0028-0836 URL http://dx.doi.org/10.1038/nature05058

[2] Squires T M and Quake S R 2005 *Rev. Mod. Phys.* **77** 977–1026 URL http://link.aps.org/doi/10.1103/RevModPhys.77.977

[3] Seemann R, Brinkmann M, Pfohl T and Herminghaus S 2012 *Rep. Prog. Phys.* **75** 016601– ISSN 0034-4885 URL http://stacks.iop.org/0034-4885/75/i=1/a=016601

[4] Zhu Y, Wu N and East C 2013 *Front Biosci* **5** 284–304 URL http://europepmc.org/abstract/MED/23277052

[5] Huebner A, Sharma S, Srisa-Art M, Hollfelder F, Edel J B and deMello A J 2008 *Lab. Chip.* **8** 1244–1254 ISSN 1473-0197 URL http://dx.doi.org/10.1039/B806405A

[6] Gokmen M T, Van Camp W, Colver P J, Bon S A F and Du Prez F E 2009 *Macromolecules* **42** 9289–9294 ISSN 0024-9297 URL http://dx.doi.org/10.1021/ma9018679

[7] Wu N, Zhu Y, Brown S, Oakeshott J, Peat T S, Surjadi R, Easton C, Leech P W and Sexton B A 2009 *Lab Chip* **9** 3391–3398 ISSN 1473-0197 URL http://dx.doi.org/10.1039/B911581A

[8] Zhao C X 2013 *Adv. Drug Deliv. Rev.* **65** 1420–1446 ISSN 0169-409X URL http://www.sciencedirect.com/science/article/pii/S0169409X13001476

[9] Duffy D C, McDonald J C, Schueller O J A and Whitesides G M 1998 *Anal. Chem.* **70** 4974–4984 ISSN 0003-2700 URL http://dx.doi.org/10.1021/ac980656z

[10] Berthier E, Young E W K and Beebe D 2012 *Lab Chip* **12** 1224–1237 ISSN 1473-0197 URL http://dx.doi.org/10.1039/C2LC20982A

[11] Utada A S, Lorenceau E, Link D R, Kaplan P D, Stone H A and Weitz D A 2005 *Science* **308** 537–541 URL http://www.sciencemag.org/content/308/5721/537.abstract

[12] Chu L Y, Utada A S, Shah R K, Kim J W and Weitz D A 2007 *Angew. Chem. Int. Ed.* **46** 8970–8974 ISSN 1521-3773 URL http://dx.doi.org/10.1002/anie.200701358

[13] Li E Q, Zhang J M and Thoroddsen S T 2014 *J. Micromech. Microeng.* **24** 015019– ISSN 0960-1317 URL http://stacks.iop.org/0960-1317/24/i=1/a=015019

[14] Terray A and Hart S J 2010 *Lab Chip* **10** 1729–1731 ISSN 1473-0197 URL http://dx.doi.org/10.1039/B927244E

[15] Benson B R, Stone H A and Prud'homme R K 2013 *Lab Chip* **13** 4507–4511 ISSN 1473-0197 URL http://dx.doi.org/10.1039/C3LC50804H

[16] Wu P, Wang Y, Luo Z, Li Y, Li M and He L 2014 *Lab Chip* **14** 795–798 ISSN 1473-0197 URL http://dx.doi.org/10.1039/C3LC51126J

[17] Chang Z, Serra C A, Bouquey M, Prat L and Hadziioannou G 2009 *Lab Chip* **9** 3007–3011 ISSN 1473-0197 URL http://dx.doi.org/10.1039/B913703C

[18] Anna S L, Bontoux N and Stone H A 2003 *Appl. Phys. Lett.* **82** 364–366 URL http://scitation.aip.org/content/aip/journal/apl/82/3/10.1063/1.1537519

[19] Lapierre F, Wu N and Zhu Y 2011 Influence of flow rate on the droplet generation process in a microfluidic chip *Proc SPIE-Int Soc Opt Eng* vol 8204 p 82040H (7pp)

[20] Kircher L, Theato P and Cameron N R 2013 *Polymer* **54** 1755–1761 ISSN 0032-3861 URL http://www.sciencedirect.com/science/article/pii/S0032386113000608

[21] Kimmins S D, Wyman P and Cameron N R 2012 *React. Funct. Polym.* **72** 947–954 ISSN 1381-5148