Topological microfluidic structures for rapid mixing of emulsions

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Abstract

A **novel use** for **topological structures** inside microfluidic channels is presented. These structures have been successfully utilised to aid in mixing of two water-in-oil emulsions in order to **force coalescence**, thereby introducing a cross linker into the primary emulsion to form particles. The method has been demonstrated by the manufacture of **self-immobilised enzyme particles**, which exhibit high activity retention while also producing **highly monodisperse** particles.

1 Background

Due to the nature of microfluidic flow, which is almost exclusively laminar, mixing is very slow and occurs primarily due to diffusion. Many ways of overcoming this limitation have been proposed [1, 2], both for active and passive mixing. A particular case of passive mixing is one in which channel surfaces are physically modified by the addition of grooves in the channel floor [3-5].
Droplet based microfluidics has received growing attention due to the many areas in which it has found application [6]. Droplets allow for the manufacture of designer emulsions [7] and the direct synthesis of particles [8]. Reagents necessary for cross linking to form particles are introduced either before droplet formation, as individual droplets requiring later merging [9], or are activated later in the process, for example by UV [10]. These schemes work well only for a limited range of flow conditions and reagents and it is difficult to scale the process for larger volume production in many applications.

A method of utilising topological micro structures for the purpose of passively mixing two streams of emulsions is explored. This introduces a novel new application for these structures.

In addition, the method of fluid introduction also allows for various time delays between the introduction of the reagents and places no limits on scale-up.

2 Design and Manufacture

Figure 1 shows a schematic of the typical microfluidic device, with the structures being positioned in the serpentine section. Standard soft lithography [11] is utilised for manufacture and all devices are manufactured from PDMS and oxygen bonded to glass slides. Channels are 70 µm deep, with the structures adding another 20 µm depth (Detail C in Figure 1). Junction A is a standard flow focusing design, with the aqueous solution (bovine serum albumin/lipase solution) being introduced in the middle 60 µm wide channel. Mineral oil is introduced into the side channels of junction A, which are 60 µm wide at the junction. Droplets are formed at this junction and flow towards junction B where a pre-emulsified cross linker (glutaradehyde and ethylenediamine) emulsion is introduced. Droplet diameter can be controlled by controlling the flow velocities of the oil and aqueous liquids at junction A, so that diameters in the range 30 – 100 µm can be produced. In this work, input flow rate is constant at 4 µl/min for the oil phase, 1 µl/min for the aqueous phase and 1 µl/min for the cross linker emulsion, resulting in final drop diameters in the 50 µm range.

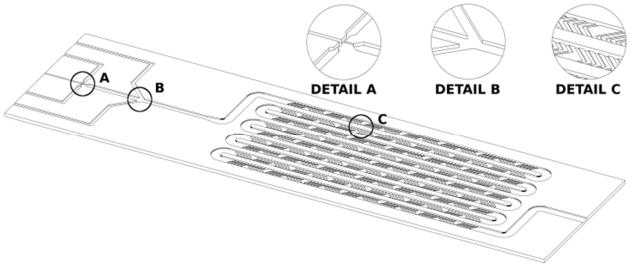

Figure 1 Schematic of the microfluidic device showing (A) flow focusing junction, (B) junction to introduce cross linker emulsion, and (C) mixing structures.

Twelve different structures have been designed and tested and an optimal structure for the mixing of emulsions has been identified. These structures are shown in **Figure 2**, where the right side shows a schematic of the designs and the left side shows photographs of the actual structures.

The dimensions of the structures, both designed and actual after manufacturing, are given in **Table 1**. Positive features on the mold become the structures adding depth to the channel, and are designed narrower than required as the manufacturing process typically adds 5% to the lateral dimension. The width of the channels in the serpentine region is 400 µm.

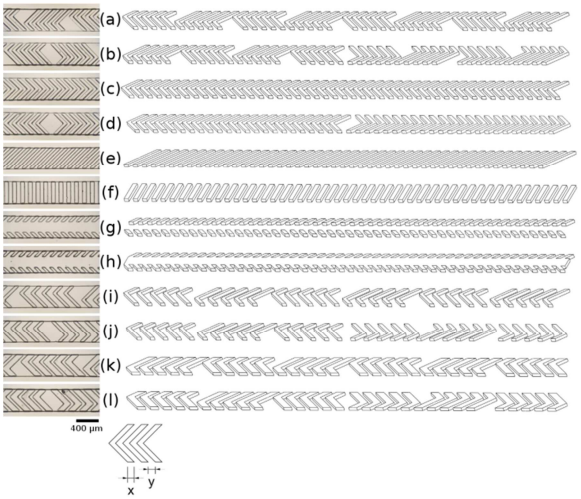

Figure 2 Schematic of the twelve tested structures (right) with photographs of the actual structures (left).

Structure	Design (µm)		Actual (µm)		Mixing Length (mm)
	x	y	x	y	
a	71	78	74	69	11
b	71	78	74	69	13
c	71	78	73	70	13
d	71	78	73	70	15
e	71	78	70	70	>26
f	71	78	73	70	>26
g	71	78	74	69	>26
h	78	71	73	70	>26
i	71	141	75	129	17
j	71	141	76	128	20
k	134	78	135	69	18
l	134	78	132	71	18

Table 1 Designed versus actual structure dimensions according to the definition in Figure 2.

3 Experimental Results

A series of microscope images of the tested structures, stitched together to form the full microfluidic channel, is shown in **Figure 3**. For clarity, the original design has been overlaid onto the experimental images. It is difficult to accurately determine when complete mixing has taken place, but it is possible to visually determine which of the structures results in effective mixing. Table 1 shows the mixing length for each channel.

Slanted (e), straight (f) and partial (g, h) structures do not work efficiently, and complete mixing is not achieved over the entire test length of 26 mm.

Reversing the structure directions (b, d, j, l) causes the larger droplets to migrate to the channel walls. These are avoided in the current work due to possible wall contamination. However, the slanted and reversed structures may be useful for applications such as sorting, although they are not considered here. Asymmetrical herringbone structures provide the best mixing (a, b, i-l), followed by the symmetric structures (c, d).

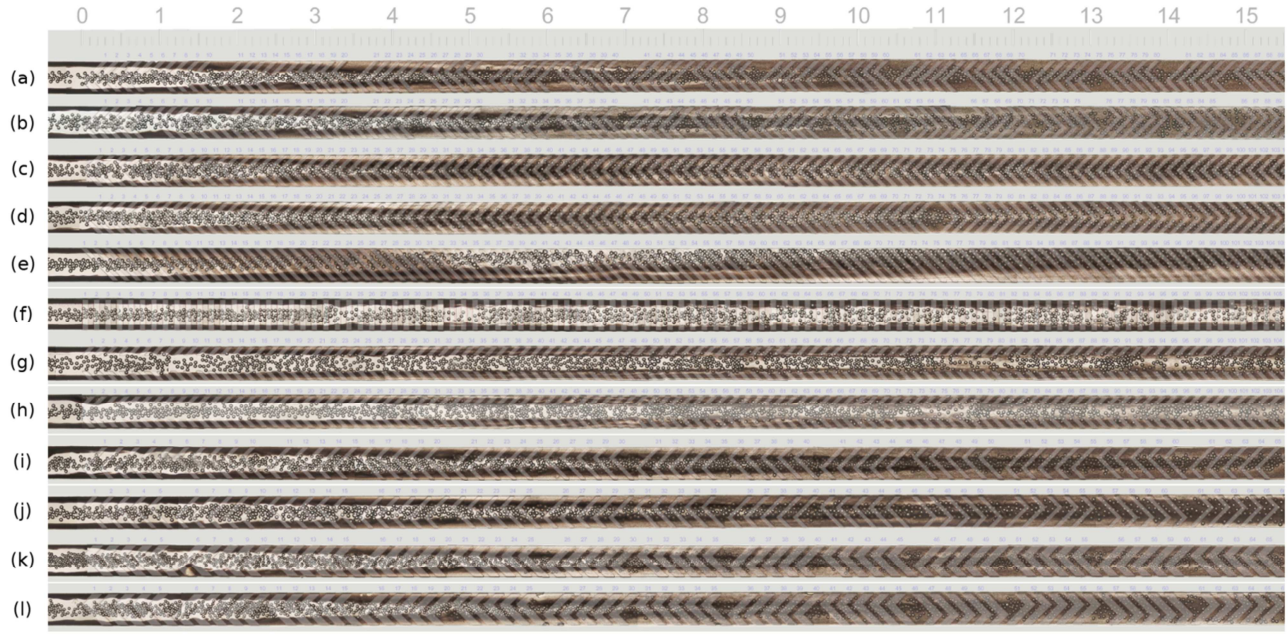

Figure 3 Microscope images of the twelve tested structures. The scale bar at the top of the image (0 – 15) is in mm. The original design is overlaid on each image to help with visualisation of the structures.

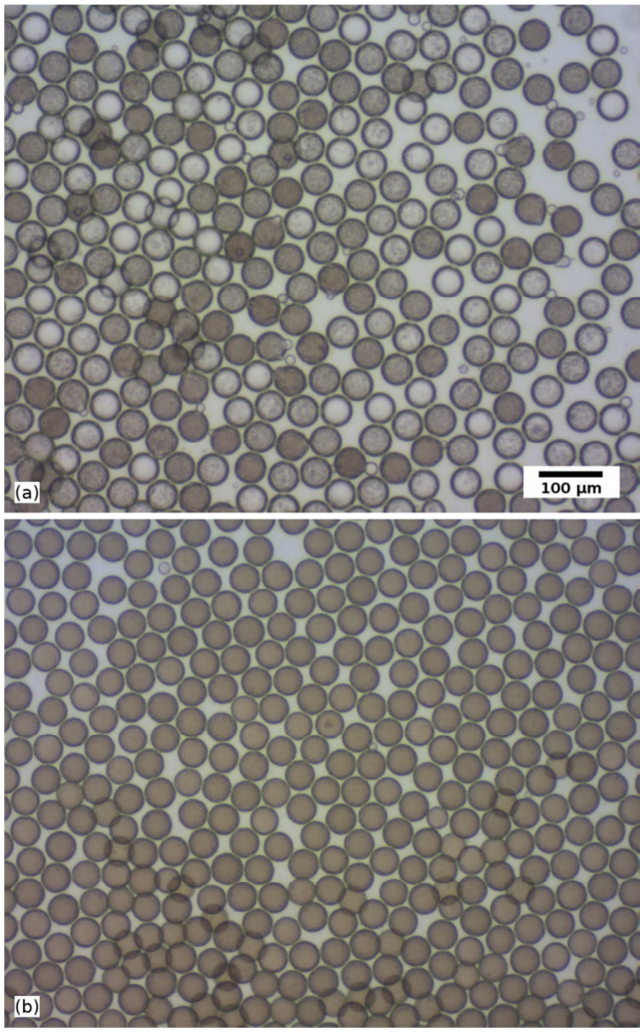

Figure 5 Photographs of droplets formed (a) with no structures compared to (b) with structures.

Increasing the distance between asymmetric structures (i, j) and increasing the structure width (k, l) both result in efficient mixing, but the mixing length increases.

In order to demonstrate the efficiency of mixing, **Figure 4** compares mixing with and without structures. Figure 4 (e) shows mixing after 104 mm in a channel without structures. Mixing is not yet complete and some droplets do not contact the cross linker emulsion sufficiently. In addition, due to the laminar flow and parabolic flow profile in the channel, droplets remain in their lateral positions, resulting in excessive cross linking or no cross linking. This is problematic for the proposed application of self-immobilising enzymes for two reasons:

1. a certain residence time is required in the microfluidic device to allow for complete mixing of the cross linker in the formed droplet, and
2. some droplets contain more cross linker than others, resulting in finished particles with different characteristics (such as stability).

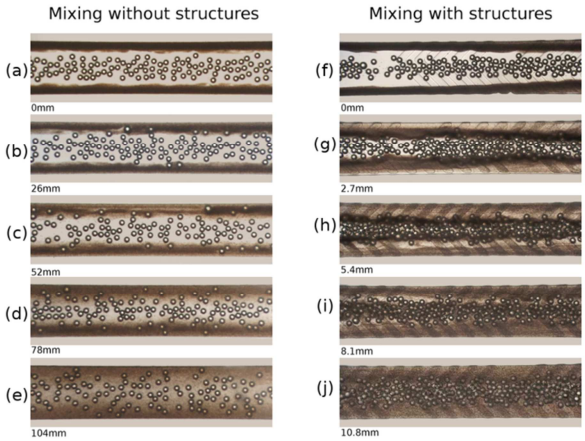

Figure 4 Experimental results of mixing with (f – j) and without (a – e) structures.

Figure 4 (j) shows complete mixing after only 10.8 mm in a channel with asymmetric structures. At the flow rates already mentioned, total residence time of the droplets in the serpentine section is 26 s. With structures, mixing is complete after 2.7 s, allowing for more than 23 s residence time for cross linker coalescence and cross linking taking place.

4 Discussion

Remarkably, it has been shown that the addition of structures results in a 10x decrease in mixing length, equating to an equivalent 10x decrease in mixing time, when compared to the same device without structures.

Rapid mixing results in longer residence time for droplets to cross link, producing evenly cross linked particles. Particle robustness has been shown by centrifuging the formed particles in order to separate them from the oil phase. Particles manufactured with structures survive this process, while particles formed without structures often coalesce or break up showing that they are not completely cross linked.

As noted earlier, the application chosen to test these structures was for the immobilisation of enzymes. Of particular importance for this application is the retention of catalytic activity of the immobilised enzyme over the free enzyme which would typically be used. Particles immobilised with the method described retain a minimum of 40 % of their activity when compared to the free enzyme, while producing particles with a diameter of 49.7 µm and a standard deviation of less than 3 %.

5 Acknowledgements

The authors wish to thank the Department of Science and Technology, South Africa for financial support, and the BMBF, Germany and NRF, South Africa for co-operation

funding. Louis Fourie and Jerry Chen are thanked for assistance with manufacturing devices.

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