HERRINGBONE LYSER: SPATIALLY OPTIMIZED MIXERS TO ENHANCE LYSING OF RED BLOOD CELLS IN WATER

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ABSTRACT

Our work is targeted to develop a chromosome total analysis system (c-tas) which can detect mutations in chromosomes by processing whole blood input. Lengthy time consuming clinical procedures are well established for each step of the process for extracting chromosomes from cells, but we would like to prepare an integrated lab-on-chip solution to capture cells, lyse them and capture individual chromosomes to analyse them for translocations. This work is directed towards developing a novel herringbone based lyser which works on the principle of mixing whole blood with pure water in time controlled fashion to lyse red blood cells (RBCs). Once the RBCs are lysed the White blood cells (WBCs) are introduced to a cell trap array so they can be captured and treated with PBS buffer to make them healthy.

INTRODUCTION

This method of lysing RBCs to isolate leukocytes is widely known as FACSlyse protocol and was recently adopted using herringbone mixers by Sethu et.al (Fig.1)[1,2]. They report that the average time taken to lyse all the RBCs in pure water is about 8-10s. This time is the time taken to mix pure water and RBCs efficiently as the lyses occurs when pure water enters the RBCs membrane and causes the membrane of RBC to rupture. Herringbone lysers utilize the principle of stretching and folding fluids to mix two fluids [3]. We have modified the design of herringbone lyser and optimized it spatially to improve the mixing and hence lysing of the RBCs.

RESULTS AND DISCUSSION

We here present 2 modified designs of the herringbone lyser as shown in (Fig. 2). COMSOL simulations of these designs show significant improvement in the mixing efficiency with both designs (Fig. 3,4). Several designs were simulated using COMSOL. Each design was further optimized to utilize the available space and improve the mixing in the microfluidic channel. The concentration/diffusion plot and the vorticity plot shows that design 3 has the best mixing efficiency and makes it optimal herringbone design for the lyser.

Initially these lysers were fabricated using CO2 laser in polymethyl methacrylate (PMMA) substrate. The layer with channels, inlets and outlets were ablated first followed by the herringbones which were laser ablated into the channels (smallest dimension ~ 250μm). To seal the device another PMMA substrate was cleaned and clamped together with the lyser and put in the oven at 108 °C for 90-120 mins. The inlets and outlets were created using modified injection needles and bonded on to the lyser using rapid setting epoxy adhesive (Fig. 5). Later optical transparency masks were also used to fabricate herringbone lysers using photolithography (smallest dimensions ~ 50 μm (Fig. 2)).

In future, we aim to integrate cell traps into the herringbone lyser to capture WBCs after the RBCs have been lysed. This will allow us to treat WBCs with PBS buffer to bring them back to their healthy state. To extract chromosomes from the healthy WBCs, we can integrate on chip reservoirs with lysis buffers or integrate electrodes in the cell traps to electrolyse the cells.

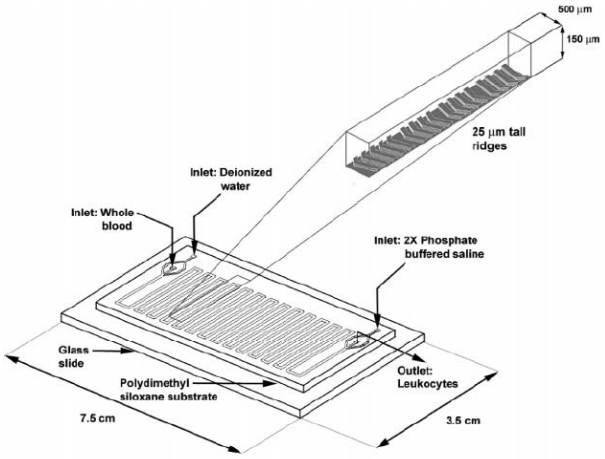
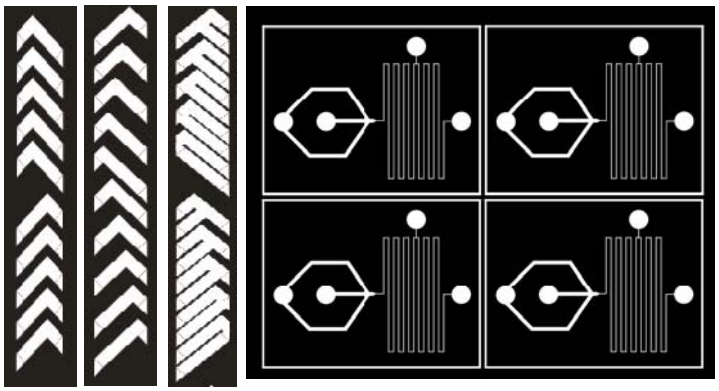


Figure 1: Rapid leukocyte isolation cassette [2]



(a) (b) (c) (d)

Figure 2: (a) Original Herringbone Design (b) & (c) modified herringbone designs I &II (d) Transparency mask for photolithography with array of lysers.

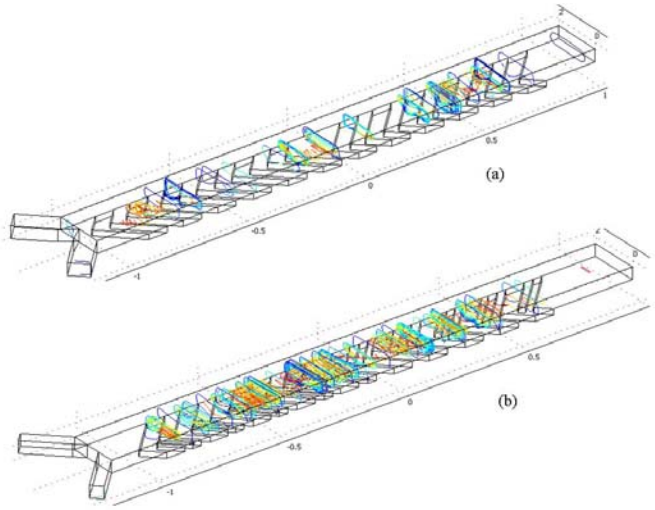


Figure 3: Vorticity Simulations for designs (a) Original (b) Modified design II.

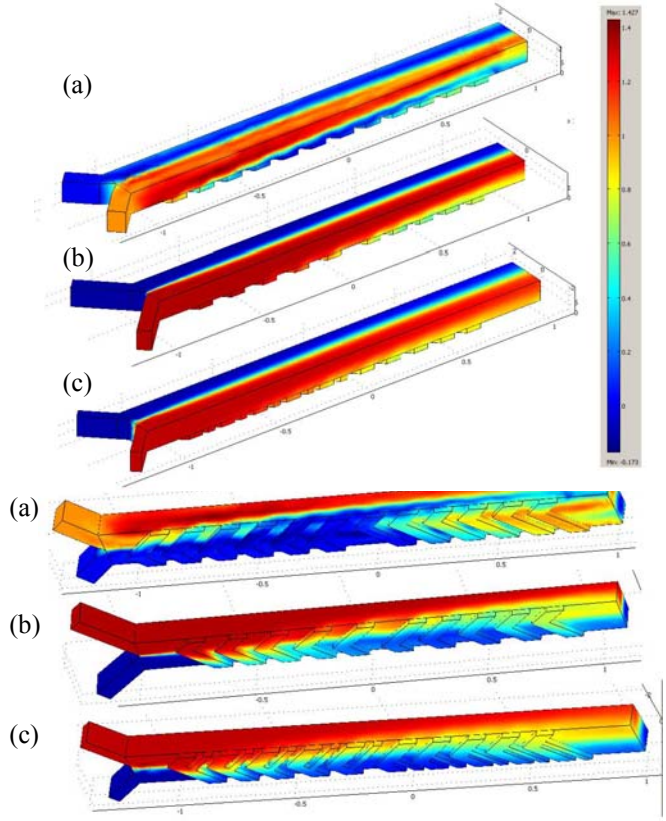


Figure 4: Concentration/ Diffusion Simulations for designs to analyze mixing efficiency top view and bottom view (a) Original (b) Modified design I (c) Modified design II.

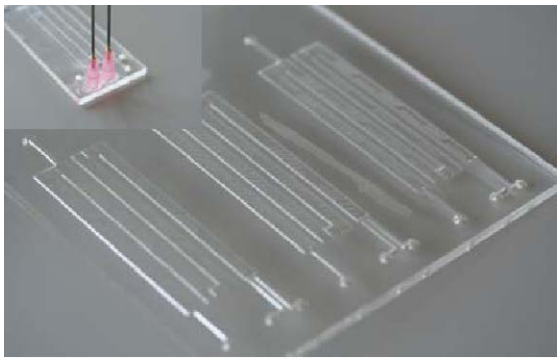


Figure 5: All 3 designs of herringbone lysers fabricated using laser ablation for mixing comparison. (Inset) Modified needles used for inlets.

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