# AN ARTIFICIAL CILIA BASED MICROMIXER FOR SUPERIOR ZEBRAFISH SPERM ACTIVATION

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## ABSTRACT

The objective of this study is to propose an artificial cilia based microfluidic device for efficient activation of zebrafish sperm by controlling the hydro-dynamics induced on the zebrafish sperm from the artificial cilia beating. The magnitude of fluid shear which has significant effect on the success of sperm activation was precisely controlled through this proposed device. A superior sperm activation of an absolute value of 70% with an average value of 65 % was achieved with a statistical significance (p-value < 0.05). The present design will advance the practice of the sperm activation significantly through the microfluidic platform.

## INTRODUCTION

Potential research use of zebrafish is immense as it ranges from modeling to human diseases and the genetic screening to screening of newly developed drugs [1-4]. Enormous usages of zebrafish resulted several thousands of zebrafish strains which are being stored in various resource centers [5]. To fulfill the huge demands for zebrafish related experiments and to activate the cryopreserved inbred strain zebrafish sperms with genetically homogeneous populations, traditionally, cell osmolality has been changed by manually mixing them with an aqueous medium. Manual mixing induces non-uniform and uncontrolled shear force on the outer membrane of the sperm cells resulting permanent damage to them. Considering the small amount of zebrafish sperms collected from each fish, there is an urgent need of an alternative technology which can yield a superior activation of the zebrafish sperm. In the recent, several passive types of micromixers have been proposed in this aforementioned context [6, 7]. Though, with these available platforms zebrafish sperm can be activated into certain extent. Still, microchannel of large length requires substantial amount of sperm samples, chemical reagents etc. Moreover, with these devices, essential fluid shear (essential factors for sperm dynamics [8]) acting on the zebrafish sperm can't be controlled precisely which further cause the detrimental effect to the outer membrane of the sperm. Through this proposed artificial cilia based microfluidic platform, the aforementioned issues will be addressed and a superior sperm activation concept can be achieved.

## MATERIAL AND METHODS

### Design layout and microfabrication flow process

The dimensional and experimental details are provided in the Fig. 1. The layout of the microfluidic platform is schematically shown in the Fig. 1 (a). The microfluidic platform consists of one inlet and one outlet and a series of artificial cilia embedded within. Layout of the device was designed in Inventor (Autodesk, Inc.) environment. As illustrated in the Fig. 2 (b), The overall fabrication process consists of a series of micro-casting process followed by polydimethylsiloxane (PDMS) molding. To get the desired mold for microfluidic structure, the created geometry was engraved onto an acrylic substrate of 5 mm thickness with the help of a conventional CNC micro-milling machine. For the mold of artificial cilium, micro-holes of 50 μm diameter with a depth of 400 μm engraved on the substrate's surface. The aspect ratio (depth/diameter) was decided to enable a high degree of artificial cilia functionality. The artificial cilium were fabricated by filling the artificial cilia composites (mixture of commercially available 5 μm neodymium-iron-boron particles (MQP-15-7, Magnequench, Singapore) and PDMS solution (Sylgard 184, Dow Corning Corp., Midland, USA) with a weight ratio of 1:4 into the mold. After filling the micro-holes for artificial cilia, the entire mold was filled with degassed PDMS. Next it had undergone a curing process by a hot plate baking at 95。C for 48 hours. The PDMS replica was peeled from the parent mold and artificial cilium were magnetized to get it actuated corresponding to the external magnetic field.

### Magnetic actuation system:

To actuate the fluid inside the microchannel through artificial cilium, external magnetic stimuli were generated by using an in-house four coil (electromagnets) system (Fig. 1 (c)) integrated with a DAQ system (NI cDAQ-9714, National Instruments, USA) and a custom-made GUI (LabVIEW 2012, National Instruments, USA). The energy was supplied to the data acquisition from an external power supply (GPR-3510HD DC Power Supply, Instek, Taiwan). The generated magnetic field with this setup can reach up to a value of 0.08 T. Time duration of the electrical current supplied to the coils for magnetic field generation was achieved with a modulated pulse width modulation (PWM) waveform of frequency 240 Hz. The rotational movement of the artificial cilium was achieved by imposing sinusoidal wave on each magnetic coil.

### PIV experiment

Sperm activation was evaluated through computer assisted sperm analysis software [9], whereas a flow visualization method was employed to investigate the underlying hydrodynamics. The relationship between rotational frequency and the trajectory of the artificial cilium was analyzed by using DLT data-viwer2 motion analysis software [10] to provide accuracy of the motion control. The micro-particle image velocimetry (μPIV) method was used to analyze the generated flow field during

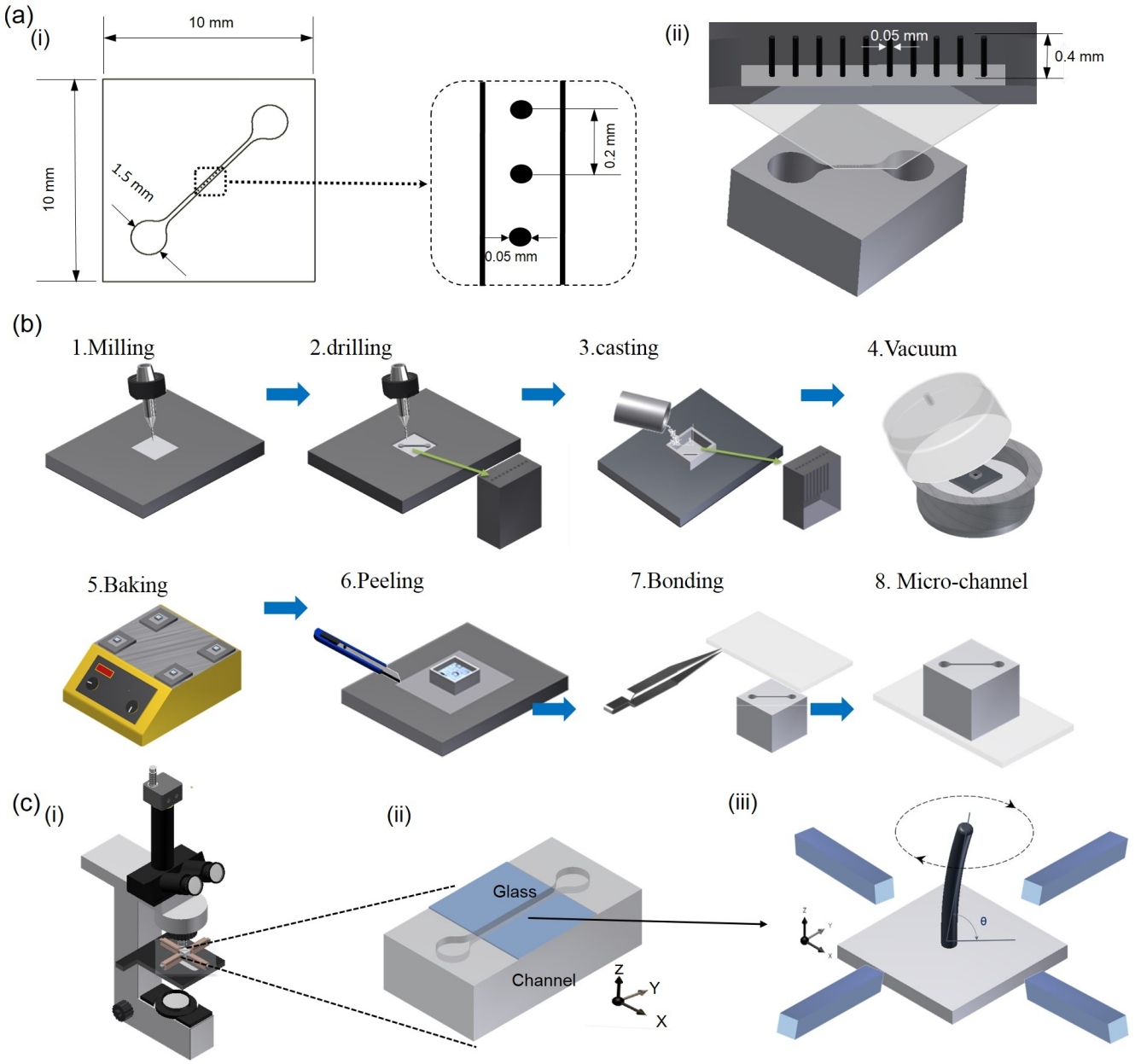


*Figure 1: (a) Schematic layout and dimensional details of the artificial cilia and microfluidic device and artificial cilia (b) Micro-fabrication process flow layout depicting a series of micro milling, drilling and PDMS casting for the fabrication of microfluidic device and artificial cilia array (c) Magnetic artificial cilia actuation system consisting of four electro magnets. The flow field was observed under a fluorescent microscope.*

the actuation of artificial cilium. For flow visualization, fluorescent polystyrene particles (Microgenics, Inc., Fremont, CA, USA) with a diameter of 3.2 µm were used. Particles motion inside the microchannel was captured by a fluorescent microscope (BX60, Olympus corp., japan). PIV software (provision, IDT, Tallahassee, FL, USA) was used to analyze the captured images. For more details about the manufacturing process and PIV experiment readers can refer our previously published articles [11, 12].

## RESULTS AND CONCLUSIONS

Zebrafish sperm activation is substantially affected by the micromixing task. So for this case, zebrafish sperm activation depends upon the device parameters such as artificial cilia rotating frequency and artificial cilia activation time period. To optimize the parameters for maximal sperm activation, experiments were carried out. From the results it was observed that more than 60% of the accounted zebrafish sperm gets activated when the rotational frequency of the artificial cilia was at a range of 1 Hz and the cilia activation time period is about 5 second (Fig. 2). With higher frequency the sperm activation

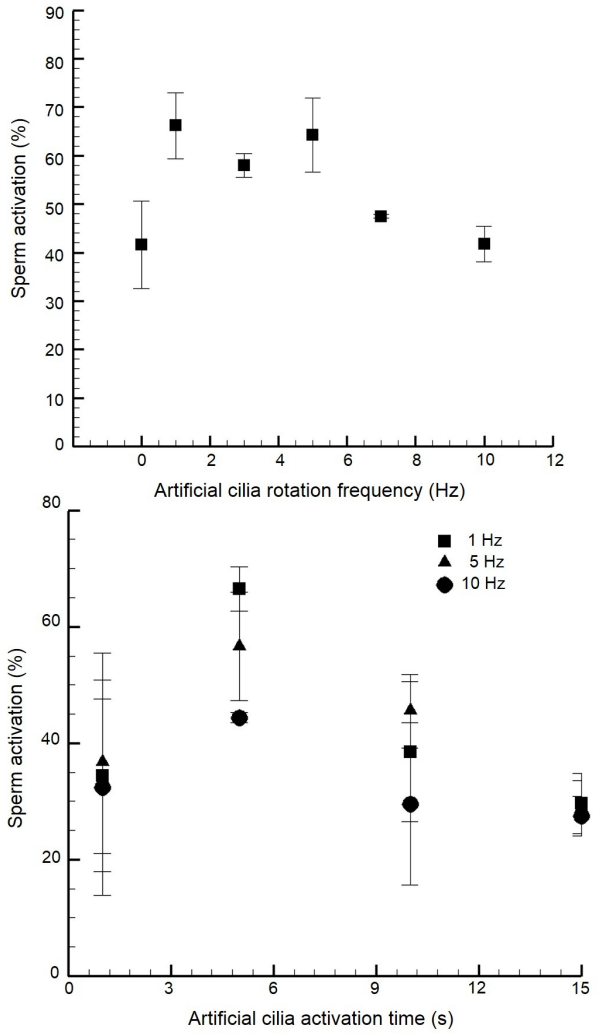


*Figure 2: Optimization of artificial cilia based micromixer parameters (a) rotational frequecy (b)Artificial cilia actiation duration towards sperm activation.*

decreases significantly which further illustrates to investigate the hydrodynamics of micro-device flow fields.

It was observed with the optimized parameters (artificial cilia rotating frequency: to 1 Hz and artificial cilia activation time period: 5 s) more than 65% of the accounted zebrafish sperm considered for the experiment were activated. Compared to other methodologies such as manual mixing, normal activation, and vortex mixer rotating at 3000 rpm, the presented method is practically beneficial with high efficiency. For instance, with this proposed microchannel, 65.5 ± 3.9% sperm motility was accounted, compared to the manual mixing technique (45.7 ± 6.5%), with a statistical significance of (n = 5, *p*-value = 0.0017) (Fig. 2).

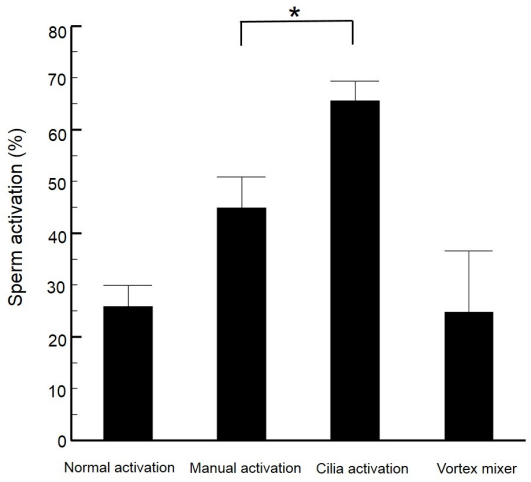


*Figure 3: Achieved sperm activation corresponding to different methodology such as normal activation, Manual activation, Cilia activation (Cilia rotating at 1 Hz) and Vortex mixer rotating at 3000 rpm.*

Moreover, it was further observed that with the increase in artificial cilia rotating frequency, oscillatory fluid shear of larger magnitude was experienced on the sperm which might be damaging the outer membrane of the zebrafish sperm. The hydrodynamic flow field obtained from μPIV analysis illustrates that corresponding to a lower frequency of 1 Hz, uniform flow field was obtained with a larger scanning area (12752 $\mu m^2$) of the artificial trajectory ensuring this superior sperm activation.

## CONCLUSION

In this work, an artificial cilia based micromixer was proposed to activate the cryopreserved sperms. Several parameters such as artificial cilia rotating frequency and artificial cilia activation time period were optimized to 1 Hz and 5 second for optimal zebrafish sperm activation, respectively.

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