DEVELOPMENT OF NOVEL MICRO MIXER AND ITS APPLICATION TO μ-IMMUNOMAGNETIC CELL SORTER

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Abstract

We report successful design, fabrication and testing of a novel lamination micro mixer to be integrated in the micro-scale immunomagnetic cell sorter (μ-IMCS), which should be a key device for future tissue engineering. This paper covers (1) the concept of μ-IMCS, (2) design and fabrication of micro mixer using lamination principle, and (3) demonstration of the mixer performance using real cells.

Keywords: Tissue engineering, Cell Sorter, Micromixer, Magnetic beads, Surface marker

1. Introduction

Tissue engineering, a field of science with a history barely spanning over 2 decades, has enabled us to produce man-made skin and cartilage. Possessing the ability to proliferate indefinitely while retaining the potential to differentiate into specialized cell types, stem cells are highly prized for both research purposes and possibly for future therapeutic applications. Unfortunately, however, they occur in minute amounts and currently there is no simple and inexpensive method to extract them from the body, e.g., bone marrow. There is clearly a need for a cell sorting device that offers high degree of purification, efficiency as well as safety. In this paper, we report the successful design, fabrication and testing of a novel lamination micro mixer to be used in the micro-scale immunomagnetic cell sorter (μ-IMCS), which should be indispensable in tissue engineering.

2. Concept and Design

Figure 1 shows the proposed concept of a μ-IMCS. Cell sample and magnetic beads are separately introduced into the device. Magnetic beads have antibody coating, which binds to a specific surface marker of the target cell. The two streams are completely mixed in the mixer, and magnetic beads attach onto target cells. Subsequently, cell-beads complexes formed are separated into the buffer fluid using an external magnetic field. Advantage of using μ-IMCS for cell sorting is two folds. Firstly, binding of magnetic beads to target cells can be achieved in seconds, which are orders of magnitudes shorter than conventional Magnetic Cell Sorters (MCS) requiring long incubation time. Secondly, unlike fluorescence-activated cell sorter (FACS)[1], we do not need bulky and expensive optical systems. This also translates to the ease of parallelization of μ-IMCS to handle larger sample volumes typically required for tissue engineering.

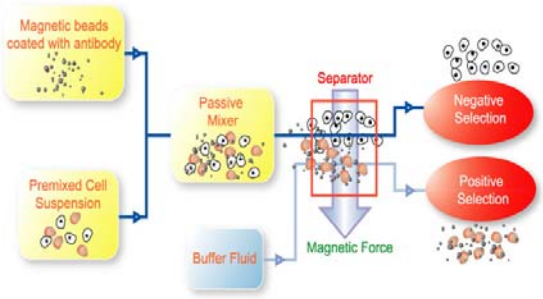

Figure 1 Concept of μ-IMCS.

Figure 2 shows the 3D geometry of a mixer unit to achieve lamination with 180 degree rotation. Unlike conventional lamination-type mixer, streams of cells and beads are inverted after each mixer unit in order to minimize sedimentation loss of cells and beads in the mixer. Corners and stagnation regions of the mixer are rounded in such a way that stagnant flow regions are reduced. It is confirmed through a CFD analysis using Fluent 6 that lamination and complete mixing are achieved (Fig. 3). It is theoretically concluded that by combining nine mixer units in series, streams of cells and beads are repeatedly interlaced with 2^9 =512 layers.

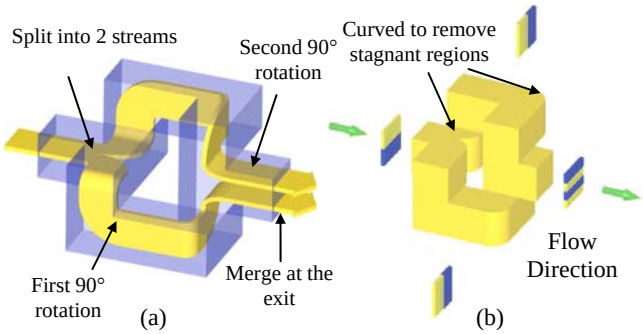

Figure 2 (a) Lamination with 180° rotation, (b) Geometry of a mixer unit.

3. Fabrication and Experimental

Soft lithography using PDMS[2] is chosen for micro fabrication of the present mixer as it offers advantages such as rapid prototyping, ease of fabrication and most importantly biocompatibility. In order to establish the mixer structure (Fig. 4), SU-8 molds are made on silicon wafer using standard lithography techniques, and three separate PDMS layers are cast from the master molds. The surfaces are treated with oxygen plasma, and permanent bonding is made after alignment of the layers, with an accuracy of ± 20 μm under microscope. The channels have a cross section of 200×200 μm^2.

Before the experiment, the mixer is first filled with phosphate buffered saline to reduce adhesion of cells to channel walls. Syringe pumps are then used to introduce the cells and Streptavidin coated magnetic beads of 1μm in diameter into the micromixer. The flow rate is chosen as 11.8 μl/min, which corresponds to a Reynolds number of unity.

4. Results and discussion

Figure 5 shows CD31 expression of Human Umbilical Vein Endothelial Cells (HUVEC) and Human Mesenchymal Stem Cells (hMSC) evaluated using a commercial MCS system. It is found that CD31 is a good indicator to distinguish between HUVEC and hMSC. Figure 6 shows cell samples including hMSC, and also HUVEC conjugated with biotin labeled anti CD31 antibody. In the present experiment, the ratio of the number of HUVEC: hMSC is chosen as 1:1.24. The corresponding volume concentration of cells and beads is 0.04% and 0.15%, respectively. Magnetic particle concentrator is used to isolate cells attached with magnetic beads from the cell sample at the outlet. Figure 7 shows HUVEC isolated with magnetic beads (Positive selection). As summarized in Table 1, the percentage of HUVEC and hMSC in the positive and negative selection is respectively 93.1% and 90.2%, which is in close accordance with the data shown in Fig. 5. An enrichment of 16.7 folds is achieved for the positive selection. The recovery yield, defined as the total number of cells obtained at the outlet divided by the number of cells introduced, is 57.3%, but much higher recovery yield should be achieved with further improvements of the mixer design.

5. Conclusions

A novel micromixer is designed and its performance is first evaluated with CFD analysis. A prototype of the μ-IMCS is fabricated using PDMS and an enrichment of 16.7 folds is achieved in a preliminary experiment using HUVEC and hMSC.

Acknowledgements

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References

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[2] B.H. Jo et al., J.MEMS, 9, 2000, pp. 76-81.

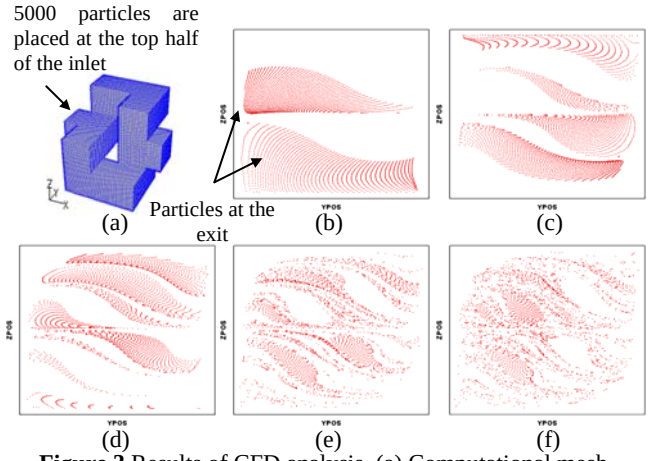


Figure 3 Results of CFD analysis, (a) Computational mesh, Particle distribution at the exit of (b) 1st , (c) 2nd , (d) 3rd , (e) 6th , and (f) 9th unit.

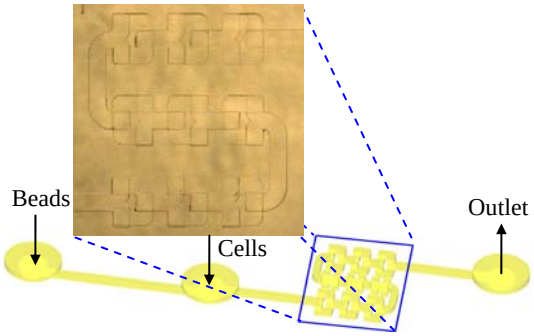
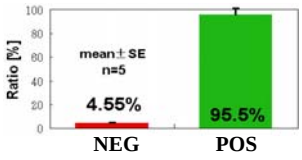


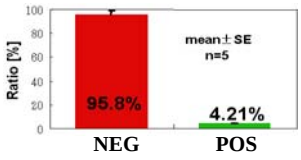
Figure 4 Mixer Test Section.

Table 1 Ratio of the cells at the outlet.

	HUVEC	hMSC	Enrichment
Positive Selection	93.1%	6.9%	16.7
Negative Selection	9.8%	90.2%	7.41



5(a) HUVEC



5(b) hMSC

Figure 5 CD31(PECAM1)expression.

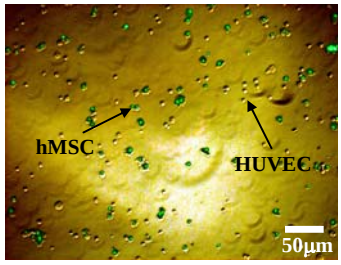


Figure 6 Mixture of HUVEC & hMSC cells in the ratio of 1:1.24.

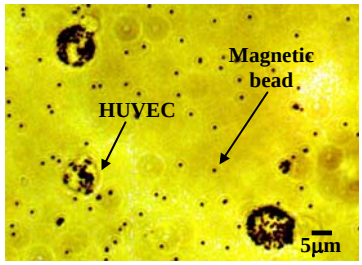


Figure 7 HUVEC cells attached with magnetic beads in the positive selection.