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Computational Fluid Dynamics Based Design of a Microfabricated Cell Capture Device

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Abstract

A microfluidic cell capture device was designed, fabricated, evaluated by numerical simulations and validated experimentally. The cell capture device was designed with a minimal footprint compartment comprising internal micropillars with the goal to obtain a compact, integrated bioanalytical system. The design of the device was accomplished by computational fluid dynamics (CFD) simulations. Various microdevice designs were rapidly prototyped in PDMS using conventional soft lithograpy technique applying micropatterned SU-8 epoxy based negative photoresist as moulding replica. The numerically modeled flow characteristics of the cell capture device was experimentally validated by tracing and microscopic recording the flow trajectories using yeast cells. Finally, we give some prospective how CFD modeling can be used in the early stage of microfluidics based cell capture device development.

Keywords: CFD, cell capture device, microfabrication, modeling

Abbreviations: CFD - Computational Fluid Dynamics, CTC - Circulating Tumor Cell, MCCD - Microfabricated Cell Capture Devices, MEMS - Microelectromechanical System, PDMS - poly-dimethylsiloxane, PIV - Particle Image Velocimetry

1. Introduction

Cell sorting is a crucial part of blood sample preparation thus has particular importance in biomedical sciences. In circulating tumor cell (CTC) research, effective cell capture is an absolute necessity, since blood represents an extremely heterogenic sample, thus reduction of complexity is of high importance (1, 2). Flow cytometry, which is the traditional way of cell sorting and capture is based on optical detection of cells encapsulated in droplets passing in front of a detector with high speed (3). The major drawbacks of flow cytometry are the requirement

for pre-sorting (filtering, centrifugation and rinsing), long processing time, need highly trained service personnel and the requirement for large sample volumes. In rare cell analysis this latter one is a limiting factor since for the time being, the detection limit of flow contemporary cytometers is around a hundred cells, while the typical number of CTCs in blood range from one (if any) to several dozen per 10 ml (4, 5). To overcome the above mentioned issues, fluorescently activated cell sorting (6), dielectrophoretic sorting (7), electrokinetic isolation, inertial separation, controlled pressure sorting (8, 9), and magnetic activated particle based (4) methods have been proposed (10). Microfabricated chips with bio-affinity surfaces represent an additional specific class of cell capture devices with the possibility of integration to further processing compartments, such as digestion, derivatization, cleaning, etc. These devices usually consist of an array of microposts coated by cell specific antibodies with the usual footprint of a microscope plate (11-13). Due to their relatively large size it is challenging to integrate them into complex lab-on-a-chip systems. The capture compartment topography could feature well-ordered pillars with uniform diameter (5, 11, 12) or randomly sized and randomly positioned posts (13).

Since microfabricated cell capture devices (MCCDs) mostly process and analyze minute amount of blood samples (14, 15), their capture efficiency is critical and could be estimated by computational fluid dynamics (CFD) approaches. Characteristic geometrical dimensions of microfabricated cell sorters and target cells are in the same order of magnitude (16), around 10 microns. Due to the narrow channels, the surface to volume ratio of microfabricated cell sorters is very high posing unusual engineering challenges, which further justify the need of computer assisted design. While numerical modeling and simulation of microfluidic systems is primarily considered as a design tool, it can also be used to support experimental data interpretation (17). From the viewpoint of bioanalysis, CFD is a developer tool to help quickly achieve an optimal design of custom made devices at low cost with a minimal number of actual experiments. PDMS is a silicon based organic polymer, which is frequently used in rapid prototyping of lab-on-a-chip or microfluidic devices due to its biocompatibility, chemical and biological resistance, transparency, easy pattern transfer and low cost (18, 19).

Numerous types of inflow liquid spreader designs have been published to aim maximized flow throughput, while keeping footprint and shear stress in the distribution channels at minimum level, and offering a uniform flow field along the device. Viovy and coworkers (20) improved

the traditional tree-like inflow design, in a way that the distribution microchannels were subsequently divided into two subchannels with equal lengths and widths. The resulted new flow distributor applied sub-channels with unequal lengths and widths according to the Hele-Shaw approximation. Please note that in this arrangement the fluid spreader took up two third of the chip footprint. Another type of microfluidic cell sorter was developed by Dickson et al. (13) with a rather simple flow distributor in which the incoming flow was equally divided to four parts, covering only 25% of the functional surface of the microdevice. In this study, a fluid distributor with an extremely small footprint was used in order to minimize the non-functional area of the MCCD. This modified disc-section shaped distributor offered lower uniformity than common channels. However, it showed no significant effect on cell capture efficiency since just the maximum value of the share rate was defined as design criteria and not its distribution.

In this paper we report on the design, microfabrication and validation of a novel minimal footprint microfabricated cell capture device with special emphasis on fluid flow engineering. First, numerous alternative chip strategies were designed by altering the size and layout of the micropillars as well as the type of flow spreader at the inlet part. The most promising designs were further investigated by means of numerical simulations. The modeled layouts were microfabricated using standard soft lithography technique, including SU-8 master replica formation followed by poly-dimethylsiloxane (PDMS) molding and oxygen plasma enhanced bonding. The flow inside the devices was validated using manual flow pattern tracking in order to evaluate the fluid dynamics performance of the developed cell sorters.

2. Modeling

The applied microfabrication using standard SU-8 photolithography and the necessary downstream processes for PDMS molding are time- and resource-consuming processes, therefore numerical simulations were applied to aid MCCD engineering. The developed CFD model was based on the laminar form of the Navier-Stokes equation, since the Reynolds number is typically around unity (16) in MCCDs:

$$\rho\left(\frac{\partial u}{\partial t} + (u \cdot \nabla)u\right) = \nabla\left(-pI + \eta(\nabla u + (\nabla u)^T)\right) \quad (1)$$

where u is the linear velocity, ρ is the fluid density, η is the fluid viscosity, t is the time, and p is the pressure. Equation 1 should be coupled to the so called continuity equation to ensure fluid consistency considering incompressible fluids:

$$\nabla \cdot u = 0 \qquad (2)$$

It was assumed, that the flowing fluid completely filled up the cell capture device (no free surface is taken into account) and had the same physical characteristics as water at 293.15^{o}K with constant dynamic viscosity (Newtonian fluid). However, blood is not absolutely Newtonian fluid due to its apparent viscosity can decrease in microchannels due to the Fahraeus effect (21), which could be the origin of some inaccuracy. Since this paper focuses on the experimental validation of the CFD based MCCD design, where water was applied as flowing media, this was neglected. Sedimentation was also not taken into account as no vertical flow was expected, therefore the MCCDs were modeled in 2D (see Figure 1).

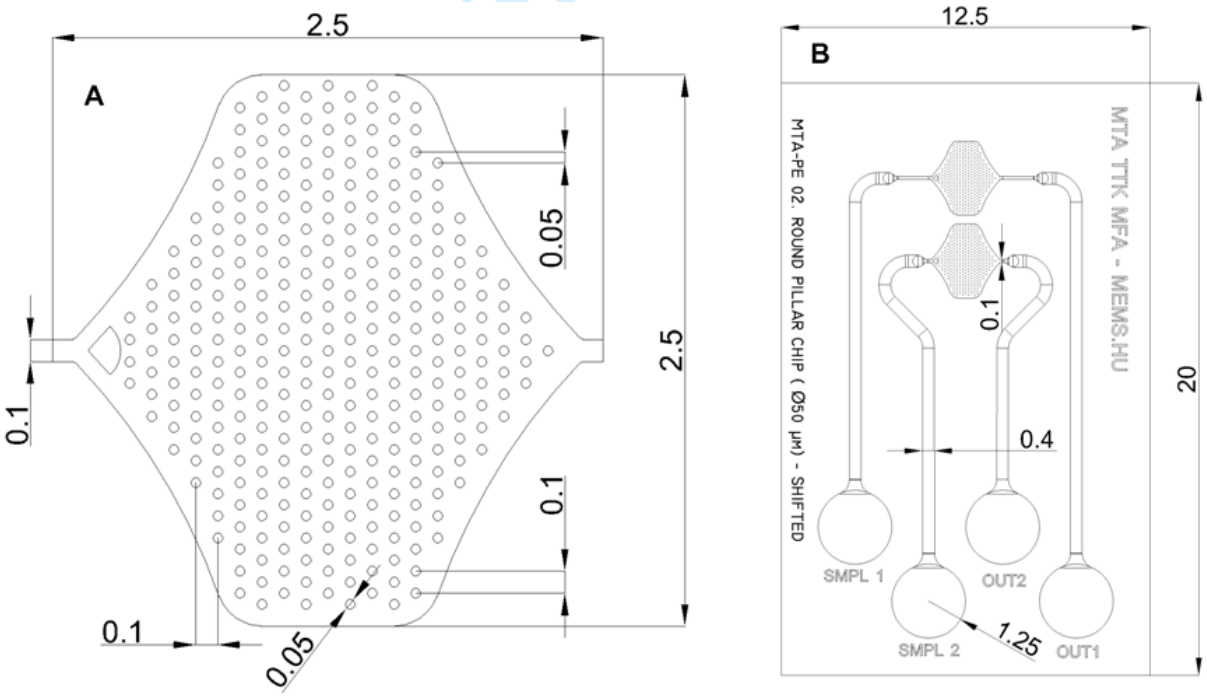

Figure 1. Schematic representation of the microfluidic cell capture device and its connections. A) The entire modeled domain with the micropillar array and inflow spreader; B) Connection channels around the functional part of the chip. All sizes are given in mm.

The inlet and outlet channels were 100 µm wide, the diameter of the micropillars were 50 µm and they were oriented as follows: each subsequent column of pillars were shifted vertically with

50 µm to ensure the highest probability of the flowing cells to interact. The major dimensions of the functional part is shown in Figure 1, panel A.

Equations 1 and 2 were solved using the finite element method based numerical solver of COMSOL Multiphysics version 4.3.0.151. Discretization (meshing) was carried out by the unmapped Delaunay triangulation technique on the bulk and the number of elements was 17494. Close to the pillars and the microdevice walls, special quadrilateral meshing was created in order to accurately resolve the flow dynamics even at high velocity gradients (5722 elements were generated). To avoid inaccuracy originated from discretization, simulations were performed several times with different meshing methods and sizes to obtain grid-independent data. The boundary conditions were defined as time invariant on all edges. The inlet (connection nearby the fluid spreader) boundary condition was represented as constant linear velocity of 1.1E-3 m/s calculated from the volume flow rate. Outlet boundary was set to atmospheric pressure using the pressure without stress condition, restricting the numerical solver to keep the pressure at a given level. This was where the flowing fluid exits the computational domain. Due to the robustness of the applied numerical stationary solver (MUMPS), just constant velocity field of 1E-3 m/s was set as initial condition in the direction of the outflow. All other boundaries were defined as no-slip-wall, i.e., velocity equals zero.

3. Materials and Methods

3.1. Microfabrication

The proposed polymer based microfluidic cell sorter structures were designed based on CFD simulation of their functional behavior and fabricated by soft lithography technique (22) utilizing SU-8 master replica for PDMS molding. First, a lithographic master was created by laser writing the optimized MCCD layout on a photoresist pre-coated hard glass substrate (Nanofilm, Westlake, CA, USA) using Heidelberg DWL 66fs laser pattern generator (Heidelberg Inst., Heidelberg, Germany). The final molding replica of the microfluidic structure was patterned by UV lithography in SU-8 3050 epoxy based negative photoresist layer (Microchem, USA) spooned on 4 inch silicon substrate, and subsequently, the crude poly-dimethylsiloxane elastomer was casted onto the master and polymerized in a clean room for 2 days. The volumetric ratio of the elastomer and the curing agent was 10:1 as specified by the vendor (Dow Corning, Sylgard 184). Then the cured PDMS was peeled, cut and bonded onto a normal

microscope slide using oxygen plasma activation of both surfaces (TerraUniversal, USA). The MCCDs were used as is, i.e. no surface modifications were applied. Since the primary engineering criteria of the cell separation subsystem was to minimize footprint while keeping its functionality, the use of integrated microfluidic connection channels were out of the scope of this study and manufactured as direct parts of the preliminary microfluidic system. Later, when the separating function itself will be a part of a multi-functional lab-on-a-chip device, the connection channels will be integrated into the system. The overall MCCD with the connection channels are shown in Figure 1, panel B.

3.2. The experimental setup

Validation of the system with experimental results is a general requirement of all modeling and simulation approaches (23). It was especially important in our case, where a quite complex geometry domain was modeled. The assembled experimental setup is depicted in Figure 2.

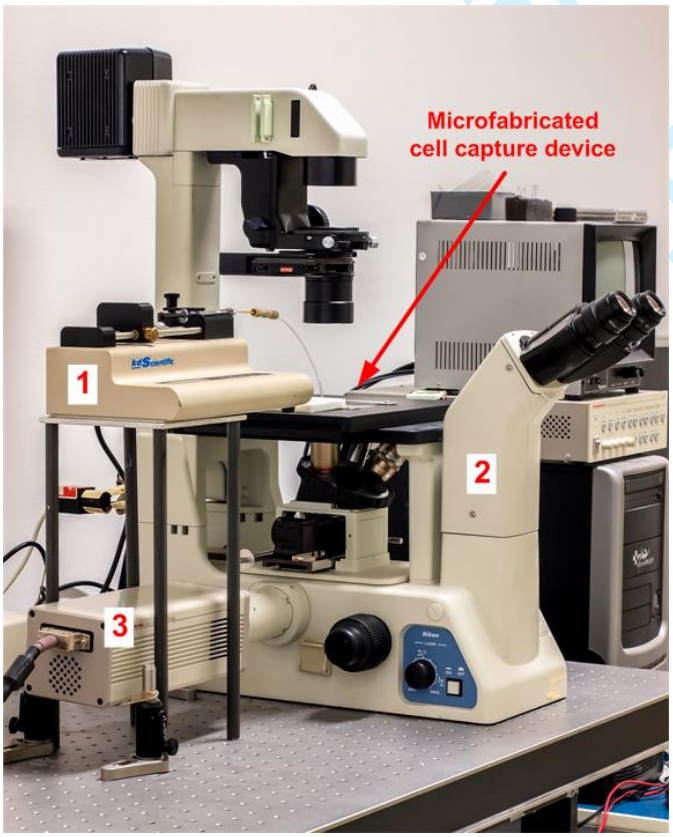

Figure 2. Experimental setup for flow dynamics validation with syringe pump (1), inverted microscope (2) and CCD camera (3).

The flow dynamics obtained by CFD simulations were validated against the experimental measurements. Particle image velocimetry (PIV) and micro-PIV are the most promising visualization techniques offering high-quality results with appropriate spatial and temporal resolutions (24, 25). The density of the usually applied polystyrene particles matches to aqueous solutions, however, their uniform surface characteristic may differ from living cells. Therefore, it was decided to use yeast cells as tracing agent in our experiments to model as close to real-world conditions as possible. Lyophilized *saccharomyces cerevisiae* cells were rehydrated in double deionized water resulting in five million cells per ml. The flow field inside the developed MCCD was traced with the model solution, which was injected by a syringe pump (KD Scientific Inc., USA) and their path was monitored and recorded using Nikon Eclipse TE200 (Nikon Instruments Inc., Japan) inverted microscope and Hamamatsu C5810 cooled CCD camera (Hamamatsu Photonics K.K, Japan). No other tracking was necessary since yeast cells were clearly visible under normal light conditions. The acquired digital video was manually evaluated and the obtained flow characteristics were statistically processed.

4. Results and discussion

4.1 Modeling

The developed numerical approach applied a laminar fluid flow model, thus a regular PC was appropriate to solve the governing equation system in couple of minutes. The calculated velocity field is shown in Figure 3.

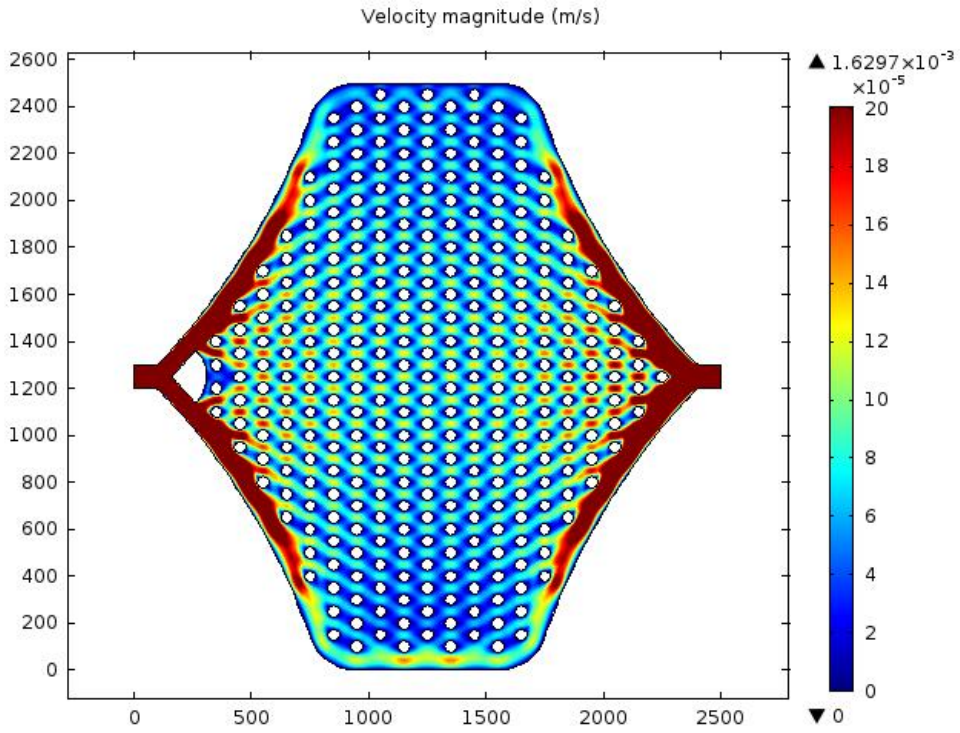

Figure 3. The calculated velocity field distribution inside the functional part of the MCCD. The warmer the color the higher the velocity.

The obtained velocity field was relatively homogenous without any fluctuation as shown in Figure 4. The fluid only accelerated at the outlet meaning that the flow distributor was smooth enough from the viewpoint of the exerted share stress, which was the linear function of the velocity according to equation 3 (26):

$$\tau_{\omega} = \frac{6\mu Q}{wh^2} \qquad (3)$$

where μ is the dynamic viscosity, Q is the flow velocity, w and h are geometry sizes of the domain of interest. The formed share stress could damage the cells in some instances (10); however, the investigation of the maximum applicable inflow velocity was out of the scope of this study.

This pillar arrangement has probably the highest flow resistance (disregard layouts where pillars are closer to each other), since the subsequent row of posts partially blocked the way for the flow. Consequently, the probability of successful cell capture is the highest with this arrangement.

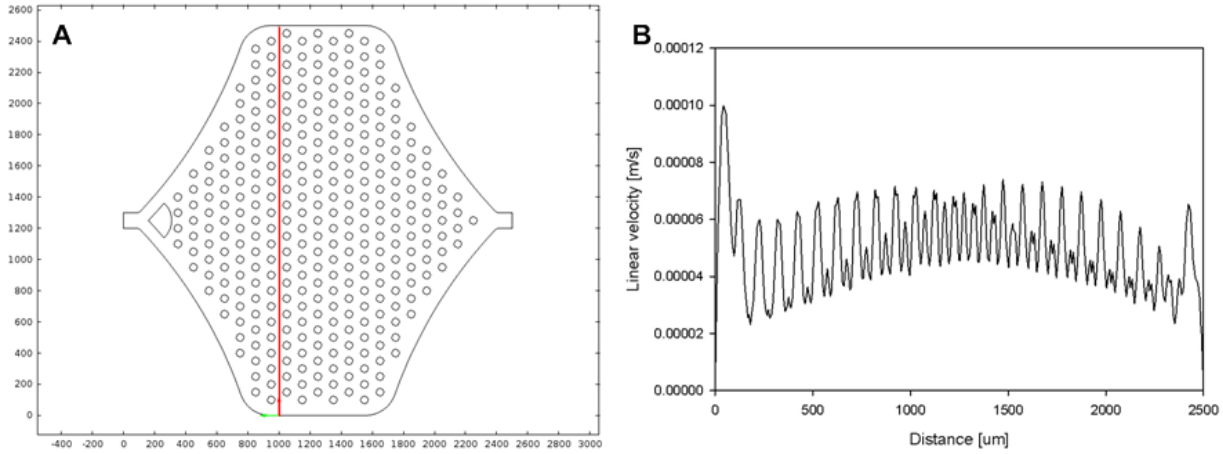

Figure 4. Calculated linear velocity was plotted at the cut line. A) The cut line after the seventh column of pillars shows where the velocity was plotted; B) Relatively uniform velocity distribution along the cut line.

This minimized footprint flow spreader will be beneficial later, when the MCCD is integrated as a crucial part of a compact bioanalytical system.

4.2 Validation

The designed and numerically modeled MCCD was microfabricated at the MEMS Laboratory of the Institute for Technical Physics and Materials Science of the Research Centre for Natural Sciences - HAS (www.mems.hu). The MCCD and its connections to the supporting tubing system were fabricated as directly connected parts as shown in Figure 5.

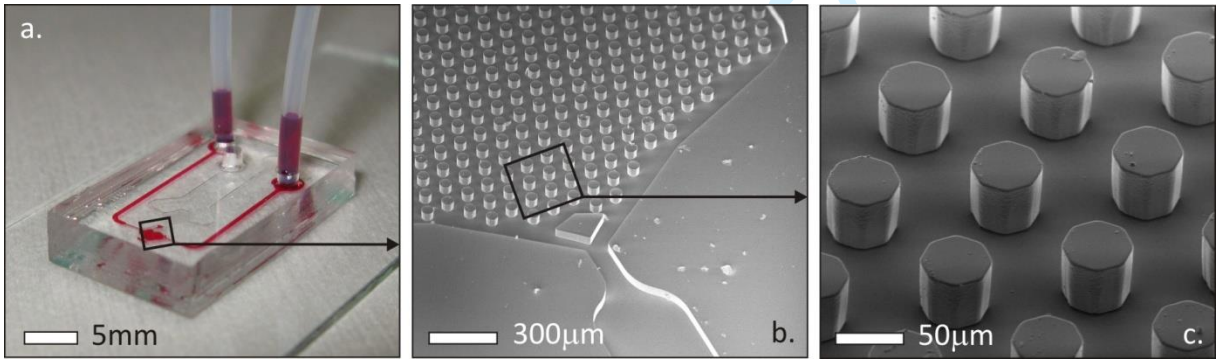

Figure 5. Cell sorter module fabricated by PDMS replica molding (a) containing MCCD layout (b) with micropillars (c).

Figure 5a shows the connection channels and the cell capture section (filled by a regular food dye). Figure 5 b and c are consequently magnified views of the posts. It can be concluded that standard photolithography followed by PDMS molding and oxygen plasma bonding is an

appropriate technique for the production of MCCDs even with very complex geometries. PDMS itself is a very soft material, which makes microfabrication (mainly peeling and cutting) easier than other substrate materials. But on the other hand, this property could be a drawback from the viewpoint of practical applications. PDMS scraps - formed due to the pipe connections - could behave like flow barrier and effect the flow dynamics inside the narrow channels of the MCCDs. Scraps can be observed even on unused devices as visible in Figure 5b and 5c.

5. Conclusions

The experimental validation of the developed model, along with the microfabricated MCCD were accomplished as reported in section 4. Yeast cells were injected into the microchip and their flow time was recorded when cells drifted along the shortest center line from the inlet to the outlet. Flow duration was measured ten times resulting in 22.1 s mean value with 0.69 s standard deviation. At this stage of the experiments, this was compared with the simulation results. Since equation 1 describes the velocity flow field as a function of time, rather than the exact position of any part of it, the COMSOL's Particle Tracing module was applied for computing the trajectory of particles in the fluidic environment. The resulted flow duration based on the CFD simulation was 21.5 s (no uncertainty), which agreed well with the experimental validation data of 22.1 s. This suggested that the developed flow model properly described the flow in MCCDs and can be used to investigate flow characteristics of novel, improved MCCDs in the early development phase.

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