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SIMULATION AND VISUALIZATION OF FLOW PATTERN IN MICROARRAYS FOR LIQUID PHASE OLIGONUCLEOTIDE AND PEPTIDE SYNTHESIS

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Abstract

Microfluidic microarrays have been developed for economical and rapid parallel synthesis of oligonucleotide and peptide libraries. For a synthesis system to be reproducible and uniform, it is crucial to have a uniform reagent delivery throughout the system. Computational fluid dynamics (CFD) is used to model and simulate the microfluidic microarrays to study geometrical effects on flow patterns. By proper design geometry, flow uniformity could be obtained in every microreactor in the microarrays.

Keywords

Microfluidic; Microarray; CFD; Flow uniformity

INTRODUCTION

A number of different microfluidic devices have gained recent interest in various fields. Examples include total chemical analysis systems or lab-on-a-chip (1), diffusion-based separation and detection devices of biological molecules (2), membraneless microfluidic fuel cells (3), microsensors (4), and ink-jet printheads (5). In addition, microfluidic microarray devices have significantly contributed to molecular biology, especially in genetic synthesis and analysis. However, several technical challenges such as speed, accuracy, sensitivity and reproducibility have been the primary obstacles in the development and usage of such devices (6). To overcome these obstacles, extensive research efforts have been done to provide a better understanding of physical phenomena and their interactions in the microscale, thereby the effects that are not common in the macroscale could be investigated and accounted for (7). Recent research examples include the designs of micropumps, microvalves, and micromixers which are usually complex and involved with three-dimensional flow behaviors. In addition, several recent studies have been focused on the significant influences of microfluidic geometries and fluid operating conditions on the successful microfluidic devices (3), (6), (8)–(12).

One of the primary applications of a microfluidic microarray in molecular biology is the genetic synthesis and analysis. DNA microarrays or biochips have revolutionized highly parallel gene expression and single nucleotide polymorphism analyses for finding and understanding the genetic causes of diseases (13) and detection of toxins in the environment (14). However,

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synthesizing large genes or DNA (over 10,000 base pairs) from arbitrary compositions with low-cost and short-time production still remains one of the formidable challenges. The large gene and other biological molecule syntheses have promising applications in several areas such as combinatorial biology, gene therapy, DNA immunization for antibody and antisera production, and gene expression. Therefore, in order to revolutionize a long gene synthesis system, microfluidic microarray devices for parallel synthesis and assembly of oligonucleotides have been developed (15)–(17).

One of the critical factors for the success of on-chip oligonucleotide parallel synthesis reaction as well as following hybridization is the flow uniformity of reagents through over thousands of parallel microreactors. Our preliminary experimental results suggested that the flow pattern and uniformity have a great impact on the synthesis yield as well as hybridization yield of surface bound probes with targets in solution. This became very obvious when our group built a first generation microfluidic microarray system, which had a simple square geometry for both the fluid delivery microchannels and synthesis microreactors (17). In this original device, highly non-uniform flow was discovered in the middle as well as corner sections of the microfluidic platform.

Since the fluid flow distribution in microfluidic devices was deemed to be a critical factor of microreactor performance, in recent years, a number of studies have been focused on this topic (12), (18)–(22). In particular, Commenge *et al.*, 2002 (12) suggested that a parallel microchannel structure may exhibit poor flow distribution uniformity which, in turn, significantly limit microreactor functions. To alleviate this problem, the effects of the geometrical dimensions of the microchannel reactors (i.e., length, width, and thickness) on the velocity distribution between channels were studied and the optimal dimensions were found. This study was carried out using an approximate pressure drop model of which fluid distribution was validated against finite-volume calculations. Further, Griffini and Gavrilidis, 2007 (18) used 2D and 3D-CFD models to study how different microchannel plate designs affected fluid flow uniformity and conclude that changing the microchannel's geometry can significantly improve the flow uniformity. In addition to these researchers' work, several studies have been done on searching for the manifold and header designs that can equalize the flow distribution in microchannels (19)–(20).

In this paper, we study the geometry effects on the flow uniformity and pressure drop in microfluidic microarray devices. A commercial computational fluid dynamics (CFD) package is used to model and simulate these systems. Several geometrical variables are investigated including microreactor depth, microchannel depth, microchannel width, and microreactor tapered inlet width and length to develop a more profound understanding of the fluid flow pattern in the microscale reactors as well as of the design of an efficient microarray geometry. New designs are proposed and proven to provide better flow uniformity than the original design.

MODELING AND SIMULATION METHOD

Computational modeling and simulation technique of a microfluidic system is composed of a mathematical model of the fluid flow and other physical phenomena. Most of the equations involved in the model are partial differential equations (PDEs) which cannot be inclusively and directly solved, especially for a complex geometry. Thus, a numerical method has to be employed to obtain the solution. To mitigate this difficulty, computational fluid dynamics (CFD) is implemented as a unique powerful tool which allows us to solve Navier-Stokes equations coupled with other physical effects such as diffusion, heat transfer, and electrokinetics. This CFD technique has recently been successfully used to design microfluidic devices, and also optimize their parameters (2)–(5), (8)–(11), (22)–(23). Several studies show

a good agreement between the CFD simulation results and experimental or analytical validations (2), (8)–(9), (11), (22).

In this study, the commercial CFD software, CFD-ACE+ by ESI CFD, Inc, Huntsville, AL, is used. Due to limited computational resources, the model's geometry is scaled down from thousands of microreactors in the microarray device to 105 square-shaped microreactors. These microreactors are divided into 7 columns and connected by 8 microchannels. The 2D plane projection of the 3D model is shown in Figure 1. The fluid flows in from the top of the model and is distributed to 4 microchannels which disperse the flow through all the microreactors. The other set of 4 microchannels collects the flow coming through the microreactors and sends the total flow out at the bottom.

After the geometry is created, meshes are generated inside all of connected-volumes which are occupied by fluid. The grid spaces are specified to be smaller in the near-wall regions than the middle of microchannels. The fluid flow problem type is then selected for steady-state with incompressible non-Newtonian fluid. The fluid properties are defined to be similar to the chemicals used in DNA synthesis experiments. The boundary conditions are determined by setting the inlet normal velocity (calculated from the known volume flow rate of 1.8 microliter/min) and the outlet pressure. No-slip wall condition is assumed. Other settings including computing schemes and parameters are assigned to defaults. However, when a calculation does not converge, the pressure computing scheme and the under-relaxation parameters are often modified. Finally, the pressure field and velocity profile are calculated. The results will be discussed in a later section of this paper.

Parametric Study

The parametric study is performed on the designed geometric variables to study their effects on the flow and pressure profiles. The geometric parameters are chosen from the basic three dimensional shapes of square microreactor and microchannel modifications which we believed could result in a more uniform flow profile. The parameters of interest which are illustrated in Figure 2 are (a) the lengths of microreactor inlet/outlet, (b) the widths of microreactor inlet/outlet, (c) the microchannel width, (d) the microreactor depth, and (e) the microchannel depth. The values of these parameters corresponding to each simulation run are shown in Table 1.

Visualization and Analysis Methods

For each of the simulation runs, the velocity contour at the middle plane of microreactor depth (shown as solid plane in Figure 2) is investigated. The flow velocities at the center of the microreactors in each column are used to evaluate the flow patterns. This is done by plotting the velocities along line probes at the center of microreactor columns and comparing them between each microreactor. Furthermore, the coefficient of variation (CV) of the highest velocities in every microreactor is also calculated. The smaller this coefficient of variation is the higher uniformity of the flow pattern.

MODEL VALIDATION

In common practice, having a fair amount of understanding over the system being considered as well as its experimental results is unquestionably a key element to a reliable utilization of the model and its simulation results. However, due to the fact that our model is a scaled down model and the corresponding experimental setup is not feasible, we are not able to directly validate the exact pressure and velocity from the model by experiment. Moreover, it is experimentally impractical to measure the pressure and velocity in every microreactor on the microarrays. Thus, in order to validate the simulation model, two types of experimental observations are instead explored and their results are compared to the model's predictions.

The designed geometry in these experiments is a simple square shape without the microreactor inlet/outlet branch which is the original prototype of our microfluidic microarray chips. The detail of the fabrications, experimental methods and results are discussed in (24).

Fluorescent Tracer Experiment

In this experiment, a microfluidic platform was mounted onto a real time chip adapter. A fluorescent dye source was connected to the adapter. Then, the fluorescent dye was pumped into the microarray, which was initially filled with isopropyl alcohol. The chip adapter was then placed under an imaging camera to take images. Figure 3 displays the line profile taken from one column of microreactors of the microfluidic device. The x-axis denotes the distance from the flow inlet along the line probe in the middle of a column of the microreactors. The y-axis represents the fluorescent intensity indicating the amount of the fluorescent dye present in the area. As can be seen, the alcohol has been replaced by fluorescent dye less in the middle region of the chip (less fluorescent intensity) than in the inlet and outlet regions. This observation indicates the inconsistency of the flow pattern from the inlet region to the middle of the device, and to the outlet region.

DNA Synthesis Chip Experiment

DNA was synthesized *in-situ* on a microfluidic device in which the microreactor depth was intentionally increased. Figure 4 shows a fluorescent image of the chip that contains fluorescent-tagged dimers arranged in a checkerboard pattern. In this design, all of the bright spots in the checkerboard pattern were supposed to be of the same intensity everywhere. However, there turned out to be a non-uniform pattern occurring in every other column where the fluid flows from left to right. In these darker regions, we believe that there is a sudden drop in the flow rate of the reagents during the synthesis; therefore, the fluorescent intensity appears lower in those areas.

Simulation Result Comparison

The experimental observations of the original designed geometry given above suggest that there are non-uniform flow patterns—very low flow rate or backward flow at the center microreactors in alternating columns. The same highly non-uniform flow in alternating columns can be seen in the simulation result as shown in Figure 5. In this figure, the velocity contour is extracted from the middle plane of microreactors. The gray color represents the velocity along the +x axis direction to the left while the black color displays the velocity in the opposite direction. The fluid flows in from the top channel, then distributes to microreactors, and finally departs through the bottom channel. If a uniform flow was achieved, the velocity contour figure would have shown an alternating pattern of gray and black columns. Nevertheless, the figure demonstrates a decrease in velocity to an inappreciable amount and even a backward flow direction in the middle of the right-flow columns which is consistent with the experimental observations. This velocity contour along a line probe in the y-axis direction at the middle of microreactors is also plotted in Figure 6. Each lobe of the plot represents a velocity profile inside a microreactor unit. The left flow velocity profile shows all positive U values. On the other hand, the right flow velocities which are supposed to be negative values for all microreactors present small positive values in the middle suggesting that there is a backward flow in that region.

SIMULATION RESULTS AND DISCUSSIONS

In this section, simulation results will be presented and discussed. The primary goal of the study is to investigate the geometrical effects on the flow profile uniformity in the microfluidic microarrays. With the model developed in this work, simulations are run according to the geometric parameter values, shown in Table 1. The velocity profile along the line probe at the

middle plane of the microreactors is extracted. The velocities in the x-axis direction (U) of all 7 microreactor columns from the model are superimposed in middle plot in Figure 7 for the nominal parameters. The uniformity of the flow profile in each individual column happens when the maximum velocities of microreactors (the peaks of each velocity lobe) to be of the same magnitude along the line probe. Additionally, the uniformity across all columns requires the velocity plots of all of the left flow columns ($+U$ values) lay on top of each other presenting only one distinct plot as well as the right flow column ($-U$ values) velocity plots exactly match each other resulting in another plot which is a negative mirror image of the left flow column plot. Because the plots in Figure 7 do not conform to the uniformity conditions, we can conclude from Figure 7 that there exist velocity differences in both the flows across columns and within the same column. Higher velocities occur in the corners of microreactors whereas the low velocities happen more in the middle of the microarray. This velocity profile is consistent with a normalized velocity distribution in 23 microchannel model in the paper by Commenge *et al.*, 2002 (12) in which the authors studied geometric effects on flow uniformity in microchannel reactors. Furthermore, similar geometric effect was observed in electric field distribution of DNA biochip study by Kassegne *et al.*, 2003 (6).

Figure 7 also shows the velocity profile when microreactor depth is varied. The top graph is the velocity plot when microreactor depth is increased while the bottom one presents the velocity profile when microreactor depth is decreased. By comparing these plots in Figure 7, the flow pattern appears more uniform between each column as well as between each microreactor row as a result of microreactor depth decreasing. Therefore, this simulation result suggests that the flow uniformity can be improved by decreasing the microreactor depth.

Besides the simulation result which microreactor depth is varied, other parameter results can be displayed with the similar type of plots. Nevertheless, comparisons of flow uniformity from velocity profile plots could be difficult, especially when the velocity bounds are not always identical. Thus, the highest absolute velocities from all 105 microreactors in the model are designated to represent the velocity profiles in each simulation run. The coefficient of variation (CV, the ratio of the standard deviation to the mean) of these velocities from the model are calculated for every simulation run in order to quantify the velocity uniformity in which low coefficient of variations imply better fluid flow uniformity. The computed coefficients of variations of this geometric parametric study are presented in Table 2.

In order to closely examine the result and sensitivities of the geometric parameters, the standard deviations in Table 2 are plotted in Figure 8. In addition, a simulation is also performed for the case of square microreactor geometry (without microreactor inlet/outlet) similar to Figure 5 with nominal microchannel and microreactor width and depth. The coefficient of variation of this case, also shown in Figure 9, is 15.76% which is significantly higher than the normal value case with the microreactor inlet/outlet. Furthermore, we can conclude that the flow uniformity could be improved by decreasing the width of microreactor inlet/outlet and the microreactor depth, and also by increasing the length of microreactor inlet/outlet channels, the microchannel width, and the microchannel depth. Moreover, the flow uniformity is most sensitive to the variations in microchannel width, microchannel depth, and microreactor depth. The uniformity, on the other hand, seems to be insensitive to the variations of the length of microreactor inlet/outlet. An example of the simulation result of the improved uniform flow geometry is illustrated in Figure 9.

By taking a closer look at Figure 9, we can see that velocity contour inside the microreactors forms an oval shape which is expected considering that the flow is in the laminar region. Therefore, there could be a flow absence from the corners of the microreactors and microchannels. So, an alternative microreactor structure design is also developed. In this prototype, the microreactor shape is circular and also the microreactor inlet/outlet is inclined

toward microchannels as shown in Figure 10. The coefficient of variation of the microreactor velocities from this circular shape microreactor model is 7.11 %.

Besides the velocity contour, the pressure profiles are as well extracted from the simulation results and investigated to estimate the flow uniformity. Figure 11 displays the pressure profile of the backflow case from Figure 5 and the uniform flow result from Figure 9. The pressure contour on the top, the highly non-uniform flow result, shows that the pressure is dispersed throughout the microfluidic platform. On the other hand, in the bottom figure of uniform flow pattern, most of the pressure drops only occur across the microreactors. This observation and the conclusion inferred earlier from the parametric study suggest that the flow uniformity could be improved by increasing the pressure drop across the microreactors relatively to the microchannels. However, some limitations in the fabrication process and operating procedure prevent us from extensively increasing the pressure drop; for example, too tiny geometry could cause a clogging problem from contaminated particles during synthesis cycles. In conclusion, our latter microarray designs have successfully provided better flow uniformity and the experimental results show that we are able to achieve uniform synthesis and hybridization in every microreactor. Figure 12 illustrates an example of one of the designs demonstrating the microfluidic oDNA array hybridized with Cy3 labeled 18-mer oligomer, developed by our research group and Xeotron Corporation, USA.

CONCLUSIONS

This study demonstrates the simulation results of fluid flow in microfluidic microarray systems for oligonucleotide and peptide synthesis. The purpose of the work is to design a new microarray platform that has the desirable flow uniformity within thousands of microreactors. The CFD results show a good agreement with the experimental observations. According to the geometrical parametric study, the microreactor depth and microchannel width are found to be the most significant effects to the flow pattern inside microfluidic platform. Consequently, the flow uniformity can be improved significantly by altering these designed geometrical variables. This indicates that by implementing the proper design of this microfluidic system, the uniform flows in all microreactor cells can be achieved. One of the later versions of our microfluidic system design, shown in the Figure 13, is the Gene chip for studying SARS-CoV virus and human host cell interaction developed by our research group and Xeotron Corporation, USA. This new microarray design provides a much better flow uniformity than the original one. Moreover, Figure 10 presents another recent design of the microarray platform in which the microreactor cells are in a circular shape. This design also exhibits a satisfactory uniform flow pattern.

Furthermore, the agreement between the experimental results and the simulations attests to the validity of the CFD technique as a modeling tool of this microfluidic system. Future work includes the transient simulations of this flow model, the oligonucleotide synthesis kinetic models, and a study of surface roughness effects on the model.

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Figure 1.
A 2D view of the 3D geometry model.

Figure 2.

One unit of the microreactor model. Top: 3D geometry of a microreactor unit, left: top view (x-y plane), and right: front view (x-z plane). The parameters are (a) length of microreactor inlet/outlet, (b) width of microreactor inlet/outlet, (c) microchannel width, (d) microreactor depth, and (e) microchannel depth.

Figure 3.
Fluorescent intensity from line probe at the middle of microreactor column.

Figure 4.
Fluorescent image of the chip containing fluorescent-tagged dimer in a checkerboard pattern.

Figure 5.
x-direction velocity contour at the middle plane of microreactors from the simulation result exhibiting a backflow profile.

Figure 6.
Velocity profile plot of Figure 5 velocity profile.

Figure 7.

Velocity profiles of the microreactor depth study result. Top: microreactor depth is increased. Middle: base case with nominal values. Bottom: microreactor depth is decreased.

Figure 8. Coefficient of variations of the highest absolute velocities from all microreactors in the model in the geometric parametric study.

Figure 9.
x-direction velocity contour at the middle plane of the microreactors exhibiting a uniform flow profile.

Figure 10.
Circular shape microreactor design.

Figure 11. Pressure contours of simulation results. Top: the non-uniform flow case; bottom: the uniform flow case.

Figure 12.

Spatially localized synthetic 18mer oligonucleotides were hybridized with Cy3 labeled complementary oligonucleotides on a microfluidic chip (half area showed). The 4 black blocks on the up part of this microfluidic chip as well as the blank cells in the chess pattern were negative controls.

Figure 13.
Gene chip for studying SARS-CoV virus and human host cell interaction.

Table 1

Geometric variable values for parametric study

Parameters/Values (μm)	-50% from nominal values	Nominal values	+50% from nominal values
Length of microreactor inlet/outlet, (a)	20	40	60
Width of microreactor inlet/outlet, (b)	25	50	75
Microchannel width, (c)	50	100	150
Microreactor depth, (d)	10	20	30
Microchannel depth, (e)	100	200	300

Table 2

Coefficient of variations of the highest absolute velocities from all microreactors in the model in the geometric parametric study

Parameters\Coefficient of variations (CV%)	Parameter -50% from nominal values	Nominal values	Parameter+50% from nominal values
Length of microreactor inlet/outlet, (a)	10.88	9.78	8.97
Width of microreactor inlet/outlet, (b)	5.17	9.78	12.82
Microchannel width, (c)	34.66	9.78	6.97
Microreactor depth, (d)	1.84	9.78	22.02
Microchannel depth, (e)	24.82	9.78	6.17