POLITECNICO
MILANO 1863

SCUOLA DI INGEGNERIA INDUSTRIALE
E DELL'INFORMAZIONE

Executive Summary of the Thesis

Computational analysis of the cell-fluid interaction in Gravitational Field-Flow Fractionation techniques

Laurea Magistrale in Biomedical Engineering - Ingegneria Biomedica

Author: Martina Guasti, Alessandra La Monica

Advisor: Prof. Maria Laura Costantino

Co-advisors: Dr. Giustina Casagrande, Dr. Debora Lattuada, Dr. Luca Possenti

Academic year: 2020-2021

1. Introduction

Considering the relatively high ratio of chromosomal birth abnormalities, it is crucial to inform parents about the possibility of fetal defects in an opportune phase of the pregnancy. Nowadays, prenatal diagnoses are mostly invasive techniques with a not negligible percentage of miscarriage, while non-invasive procedures are mainly screening tests that cannot be used as an ultimate detection of fetal disease. However, the discovery of circulating nucleated fetal cells in the mother's blood has been the turning point for non-invasive prenatal diagnoses. The most promising cells for this application are fetal erythroblasts, which could be isolated through an innovative separation approach based on a Gravitational Field-Flow Fractionation (GrFFF) method. This method, applied to the Lattuada microfluidic channel, combines the effects of the flow of the operating fluid to the gravitational force in order to obtain the separation of the cells of interest without altering nor damaging them.
The aim of the proposed study is focused on an accurate description of the physics behind the above-mentioned problem. Specifically, the spotlight is on modelling cells behaviour in terms of deformation and interaction with the fluid flowing inside the channel. Therefore, the present work is based on the development of a multi-phase computational model that describes the cell's transport through the microfluidic channel.

2. Modeling and Validation

At first, the channel has been modelled with the 2D and 3D representations, adopting the actual dimensions of the experimental one. Since the channel width is noticeably higher than its height, we could apply the "two semi-infinite slabs" theory to this geometry. We tested the two schematizations to verify this geometrical hypothesis through different passages: firstly, we checked the achievement of a parabolic velocity profile along the channel height; then, we ensured that the maximum velocity was obtained at half-height with a magnitude of 3/2 the average speed; lastly, we made sure that the flow was fully developed at the position of cells insertion. After the validation of the model, we have considered for further evaluations a reduction of both geometries, taking into account exclusively the sections of interest to decrease the computational cost required. We tested different

mesh sizes in steady-state conditions and different time step dimensions in transient conditions, intending to achieve the most acceptable balance between solution accuracy and computational cost. Since the results in the optimisation phase were satisfactory, we proceeded with the finite volume multi-phase model.

Volume of Fluid model

Subsequently, we've implemented the Volume of Fluid (VOF) model to track the fluid-fluid interface between the two fluid phases: medium and cell. We used the reduced 2D schematization to optimize the model thanks to its low computational cost with respect to the 3D channel. Then, for further verifications, we compared the 2D and 3D solutions. We established the channel height based on the experimental set up, equal to 200 μm, together with the flow rate magnitude of 250 μl/min, corresponding to an average velocity of 1.04 mm/s.
Starting from the data found in the literature, we've chosen 10 μm as a standardized diameter of the cell. Then, we have set the following materials' properties: 1006 kg/m^3 and 1073 kg/m^3 as medium and cell density respectively; 0.001 Pa*s as the medium viscosity, 3×10^{-5} N/m as the value for the surface tension between the two phases. Additionally, to maintain the circularity of the cell we imposed a contact angle of 179° between the two fluids. After that, we've initially chosen the most suitable value based on average speed for cell viscosity, set as 0.2 Pa*s, even if literature data are discordant in this regard. Noticing that this parameter was particularly influential on the model solution, we carried out subsequent studies. We established the mesh dimensions based on the most satisfactory found in the previous analysis, corresponding to 2×10^{-3} mm, corresponding to the best balance between computational cost, simulation time and accuracy. We employed the implicit scheme for a better convergence of the solution, so the constraints about the time step size were limited to the mesh dimension. Afterwards, we tested different time steps (0.0001 s, 0.001 s, 0.01 s) to discover the best compromise between computational cost and accuracy of the solution.
We then evaluated the velocity trends and decided to exclude the one relative to the greater dimension (0.01 s) since it didn't represent the actual course of cell transport and was associated with a significantly lower solution accuracy. In the end, 0.001 s has been identified as the most reasonable size.

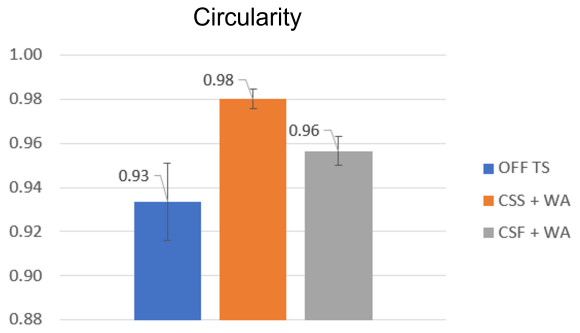

Figure 1: Cell circularity values for CSS, CSF models and for no model activated (OFF TS).

Another fundamental part of the optimisation process has concerned the choice of the interaction model to represent the surface tension between the two phases. There are two available models: *Continuum Surface Force* (CSF) and *Continuum Surface Stress* (CSS). We compared the velocity and displacement trends of the cell, together with its deformation, for both of them (avoiding cells to adhere to the channel bottom by setting the Wall Adhesion command and imposing a contact angle of 179°) as well as for no model activated (OFF TS).
The CSS model better allowed the cell to maintain the circular shape without high deformations; the comparison of the circularity results, calculated as $4\pi Area/Perimeter^2$, is visible in Figure 1. Moreover, the CSF model introduces a forcing term in the continuum equation, therefore, it probably affected the cell motion giving a lower velocity than the one obtained with the CSS model, as shown in Figure 2. In these simulations, we set a cell viscosity of 0.43 Pa*s, based on *Fabry et. al* (2003) study, which was slightly higher than the previous one. Then, we compared the velocity of each simulation with the theoretical value of cell speed, which was obtained from the equation corresponding to the parabolic velocity profile developed within the channel. However, all the velocities reached were incredible lower than the theoretical value that the cell should assume in its position. So, it's noticeable that a sensitivity analysis to examine

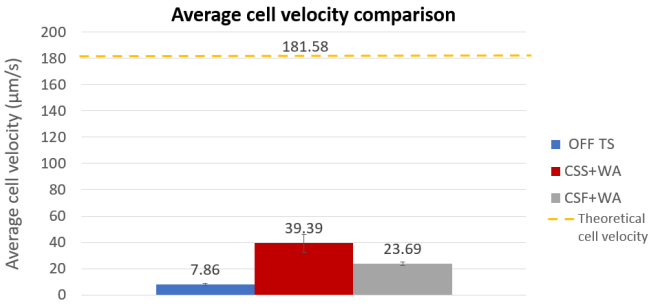

Figure 2: Cell speed magnitude for different interaction models.

the influence of all the input parameters should be done.
Then, after the optimisation of all the aspects that could influence the solution, we compared the 2D and 3D models imposing the same input parameters. We evaluated the velocity and the displacement trends and verified that the velocity magnitude achieved in both simulations was similar and comparable with the theoretical speed. The latter was estimated from the parabolic equation of the velocity profile, based on the position that the cell should assume in the channel height. Additional studies could be required to reach a better match between the aforementioned results of the two examined channels.

3. Input parameters sensitivity

The sensitivity analysis on the input parameters represents one of the fundamental phases of the proposed study; indeed, by evaluating each parameter's influence on the model, we established which are the relevant factors to control and define accurately. Firstly, we chose the input parameters subject of the analysis, then based on the literature, we determined the reference values to implement the standard simulations. According to those standard values we designated their upper and lower variations to obtain a range consistent with the literature. In Table 1, we set the standard values and their variations. Among all the parameters, the cell viscosity was particularly critical: the literature reports very heterogeneous data, ranging from very high values such as 100-200 Pa*s for neutrophils to significantly lower values, up to 0.003 Pa*s for red blood cells [5–8].

Input Parameters			
Factors	**Inf**	**Std**	**Sup**
$\rho_{cell}\ [kg/m^3]$	1073	1100	1130
$\mu_{cell}\ [Pa*s]$	0.043	0.43	4.3
$\tilde{\mu_{cell}}\ [Pa*s]$	0.22	0.43	0.86
$\sigma\ [N/m]$	$2{\times}10^{-5}$	$3{\times}10^{-5}$	$4{\times}10^{-5}$
$\phi_{cell}\ [\mu m]$	7	10	13
$h_{channel}\ [\mu m]$	100	200	400
$v_{in}\ [mm/s]$	0.52	1.04	2.08

Table 1: Inferior, standard and superior values for each parameters.

From the previous analyses, we've found an optimal viscosity of 10^{-1} Pa*s order of magnitude, therefore, we looked for values belonging to that range. Based on the *Fabry et al.* (2003) study, we set 0.43 Pa*s as the reference value of viscosity. To carry out the analysis, we implemented 12 simulations, with Δt=0.001 s, changing one parameter at a time and maintaining the others to the reference values. In this way, we detected the influence of each factor: to do so, we evaluated the sensitivity analysis outputs, specifically cell x-position and its average velocity. Although other parameters such as surface tension or cell diameters have a certain impact on those output data, the most decisive effect is due to the viscosity. In Figure 3 below, we reported the cell mean velocity, comparing the effect of each parameter value change. Viscosity affects the solution, changing the output values even of an order of magnitude. Based on those results, we decided to detect the optimal viscosity value for this model to ensure the reliability of the outcome.

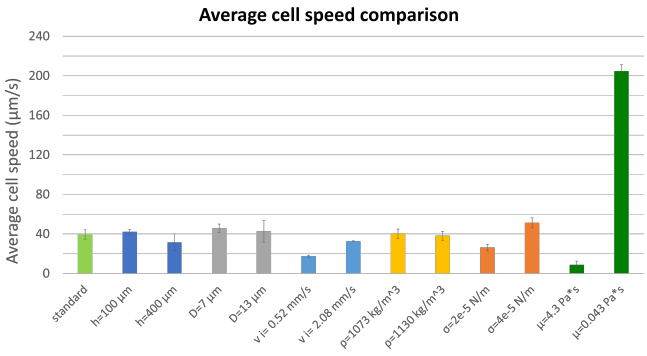

Figure 3: Comparison of each parameter influence on average cell velocity.

4. Inverse problem

The sensitivity analysis has shown the importance of viscosity on the proposed model. So, we've decided to search for those values that would guarantee the achievement of reliable results with respect to experimental data, implementing an inverse problem. We've chosen monocytes population data owing to their easy isolation process and their round shape. Those data were obtained by a channel 225 μm high with the adoption of a 350 μl/min flow rate, corresponding to an average fluid velocity of 1.3 mm/s, so we deployed those changes into the computational model, maintaining the same properties of medium and cell as before.
The experimental monocyte data have been extracted from a 10 s video of the cells flowing into the channel; in particular, through a mixed computational/empirical approach, it has been possible to identify the respective exit time between the 13th and 17th minutes. From the experimental data, we took the average cell diameters and the associated average velocity corresponding to each minute, from the 13th to the 17th. Although they respected the foreseen exit trend, we decided to interpolate with a 2nd-order polynomial function mean diameters and corresponding cell speeds for better reliability. Based on those interpolated values, the optimal viscosity would have been achieved for each considered minute when the simulation returned a computational average velocity in line with the experimental one, taking into account an allowed error of 2%.
The respective data about adopted diameters, goal velocity values and obtained velocities were reported in Table 2.

Min.	$D_{cell}(\mu\mathbf{m})$	$v_{target}(\mu\mathbf{m/s})$	$v_{avg}(\mu\mathbf{m/s})$	**Err**
13	14.30	253.94	256.26	0.91%
14	12.64	206.07	207.54	0.71%
15	11.40	183.50	185.27	0.97%
16	10.57	174.78	177.89	1.78 %
17	10.16	172.32	170.31	1.16 %

Table 2: Diameters (D_{cell}) and target velocities (v_{target}) from interpolation of experimental data, average cell velocity obtained from inverse problem (v_{avg}) and relative error (Err) for each considered minute.

As shown in Figure 4, the results obtained from the inverse problem showed the existence of a correlation between the viscosity value and the cell dimension; specifically, as the latter increases, the viscosity tends to decrease. Considering the viscosity value of RBC cytoplasm, considerably lower than our results, we hypothesized that this relation could depend on the percentage of nucleus inside the cell. Therefore, we reported in Figure 5 the correlation between the % of nucleus and cell diameters for the entire monocytes population. We identified through a power trendline a decreasing trend, as a validation of what was said before. Subsequently, among the experimental data, we considered the ones corresponding to cells having diameters greater than 10 μm. Besides, we divided those data into five intervals then we obtained the average values in order to have diameters matching the ones used in the inverse problem.

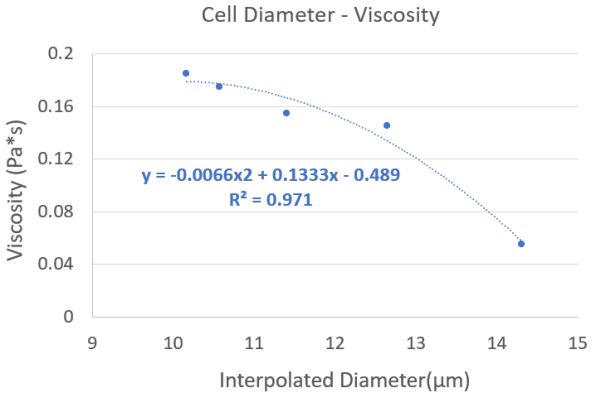

Figure 4: Viscosity values obtained as a function of cell diameters with a second degree interpolation polynomial.

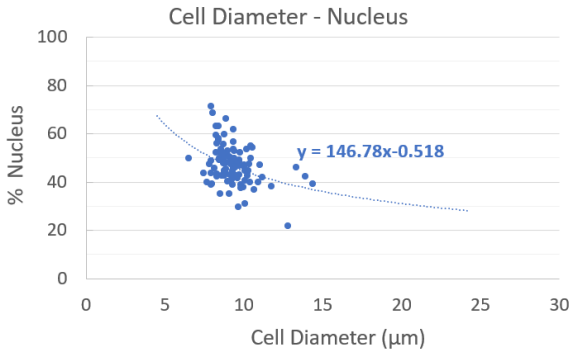

Figure 5: % nucleus - diameter for all cellular population: decreasing trend obtained by setting a power curve with its function expressed in the graph.

Viscosity considerations

From the observed results, it's reasonable to say that the viscosity of the nucleus has a substantial influence on cells with a higher nucleus percentage, hence smaller total dimensions, whereas it has minor importance in cells with a larger diameter in which there is a considerable influence of the cytoplasm viscosity. Therefore, the global viscosity should include the contribution of both parts.
To verify this hypothesis, we calculated through the following equation the total viscosity of the cell as a weighted average between the two values of cytoplasm and nucleus; as cytoplasm value, we adopted the lower bound of the viscosity range found in the literature: 0.003 - 0.01 Pa*s [3]. Anyhow, since general data for the nucleus are extremely heterogeneous, we decided to consider a value from exclusively in vitro studies on the determination of nucleus viscosity, which are instead sufficiently aligned [1, 2, 4]. So, the final employed values are respectively: $\mu_{cyt.}$=0.003 Pa*s and $\mu_{nucleus}$=0.51 Pa*s.

$$\mu_{tot} = \frac{\mu_{cyt.} \times \%_{cyt.} + \mu_{nucleus} \times \%_{nucleus}}{\%_{nucleus} + \%_{cyt.}}$$

Then, we compared the overall viscosity values obtained for each minute with the ones found from the inverse problem. The results can be seen in Table 3.

Min.	D_{cell} **(μm)**	μ_{ipb} **(Pa*s)**	μ_{tot} **(Pa*s)**
13	14.30	0.06	0.21
14	12.64	0.15	0.22
15	11.40	0.16	0.22
16	10.57	0.18	0.24
17	10.16	0.19	0.23

Table 3: Viscosity obtained from the inverse problem (μ_{ipb}) and viscosity calculated through the weighted average (μ_{tot}).

5. General discussion

In the present work, we defined the optimal parameters, such as mesh dimension, time step size and interaction model, for the proposed computational model; as the complexity of the problem increases, more accurate and deepened evaluations should be carried out.
From the comparison between 2D and 3D models, we observed that tridimensional results were more affected by the mesh dimensions. In particular, the looser mesh adopted due to computational constraints slightly altered the 3D solution. Despite this, we found comparable outcomes; also, we didn't achieve any additional information through the 3D geometry. So, considering the high computational cost associated, we choose the bidimensional one for further analysis.
To pick the most suitable interaction model, we looked for the most conservative one that did not introduce any constraint. The CSS model has been recognized as the more satisfactory one, even though it showed an evident velocity dependency on the viscosity value.
So, noticing the difference in the results obtained with different cell viscosity values, we decided to implement a sensitivity analysis of input parameters. From its outcome, we verified the significant influence of the viscosity value on the model solution. We point out that the results obtained are strongly affected by the input parameters and their upper and lower variations choice. Therefore, these results are specific for this application, and different input ranges might lead to different conclusions.
Proved the crucial importance of cell viscosity for the proposed work, we implemented an inverse problem that allowed the detection of information on the viscosity influence, starting from the measurements of its effects on the model solution. Since we used cell diameters and velocities from experimental data to find the optimal viscosity value, they were strongly affected by high standard deviations that could hardly be reduced. Therefore, we decided to use a 2nd-degree polynomial function to interpolate them and reduce the variability introduced by experimental tests. Specifically, the main reason was the need of representing a continuous phenomenon of which we have only a discrete evaluation. We had only 5 available data relating to minutes 13-17, so we preferred to use data coming from a continuous function that better describes the total physical happening.
Although the outcome of the inverse problem was comparable to the calculation of the total viscosity, we noticed some discrepancies probably caused by the dispersion of the experimental data. In particular, the velocity associated with minute 13 was remarkably higher than the

others. So, for the computation of the inverse problem, it has been required a lower viscosity to obtain that result; this restricting request has led to a final value that was not in line with the other ones. Nevertheless, following those outcomes, we proved the increasing importance of nucleus viscosity with the decreasing cell dimensions significantly affecting the total viscosity of the cell.

6. Conclusions

In the present study, we developed a finite volume model to represent the cell as a fluid phase immersed and transported within a fluid. The cell movement has been reproduced, with no meaningful deformation seen during its transport. The optimal mesh dimension concerning computational cost and solution accuracy has been identified as 2×10^{-3} mm, together with 1×10^{-3} s as the optimal time step, based on the cell displacement and speed. Then, to better reproduce the surface tension and the interaction between the two phases, the CSS model has been defined as the optimal one, although the associated velocity was different from the theoretical one and strongly influenced by the viscosity value.
The outcome of the sensitivity analysis on input parameters revealed that the viscosity of the cell phase had a significant influence on the model solution. So, to find the most suitable value based on average cell speed, we implemented an inverse problem, whose outcomes allowed the individuation of the contribution of both nucleus and cytoplasm viscosity on the total one, comparable with results obtained from experimental data. Those evaluations granted the refinement of the model and laid the foundations for further future developments. For example, some potential evolutions of the proposed work could be considering two (or more) cells inserted in the channel and evaluating their interaction or motion through space. Otherwise, following the total viscosity considerations, it could also be possible to introduce a third phase representing the cell nucleus within the cytoplasm to evaluate their movement and interaction.
This work represents a step towards the individuation of optimal parameters of the under consideration channel, making the separation method reliable, repeatable and safe.

References

[1] Christina M. Caragine, Shannon C. Haley, and Alexandra Zidovska. Surface Fluctuations and Coalescence of Nucleolar Droplets in the Human Cell Nucleus. *Physical Review Letters*, 121(14):148101, 2018.

[2] Alfredo Celedon, Christopher M. Hale, and Denis Wirtz. Magnetic manipulation of nanorods in the nucleus of living cells. *Biophysical Journal*, 101(8):1880–1886, 2011.

[3] Mike de Haan, Gabor Zavodszky, Victor Azizi, and Alfons G. Hoekstra. Numerical investigation of the effects of red blood cell cytoplasmic viscosity contrasts on single cell and bulk transport behaviour. *Applied Sciences (Switzerland)*, 8(9):1–17, 2018.

[4] Anthony H.B. De Vries, Bea E. Krenn, Roel Van Driel, Vinod Subramaniam, and Johannes S. Kanger. Direct observation of nanomechanical properties of chromatin in living cells. *Nano Letters*, 7(5):1424–1427, 2007.

[5] R. M. Hochmuth, H. P. Ting-Beall, B. B. Beaty, D. Needham, and R. Tran-Son-Tay. Viscosity of passive human neutrophils undergoing small deformations. *Biophysical Journal*, 64(5):1596–1601, 1993.

[6] C. T. Lim, E. H. Zhou, and S. T. Quek. Mechanical models for living cells - A review. *Journal of Biomechanics*, 39(2):195–216, 2006.

[7] D. Needham and R. M. Hochmuth. Rapid flow of passive neutrophils into a 4 μm pipet and measurement of cytoplasmic viscosity. *Journal of Biomechanical Engineering*, 112(3):269–276, 1990.

[8] M. A. Tsai, R. S. Frank, and R. E. Waugh. Passive mechanical behavior of human neutrophils: effect of cytochalasin B. *Biophysical Journal*, 66(6):2166–2172, 1994.