MULTIPHYSICAL SIMULATION OF HYDRODYNAMIC TRAPPING OF *S. cerevisiae*

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Abstract

The yeast *Saccharomyces cerevisiae* has been used for the study of aging in eukaryotic cells. The traditional method for this study uses micromanipulators, which makes it difficult, time-consuming, and expensive. Over time, different microfluidic platforms, generally referred to as "mother machines," have emerged to facilitate this process, making RLS (Replicative Life Span) measurements. However, these methods use mechanical trapping, which puts pressure on the cell, which can affect its physiology (growth rate, gene expression, aging, etc.). To avoid this, we designed a new microfluidic platform that does not use mechanical but hydrodynamic trapping, with the aim of trapping the mother cell so that the daughter cells can separate. Using COMSOL Multiphysics® software, we designed and simulated a geometry to analyze different parameters in mother cell trapping in the mother machine. The device consists of a channel that has an inlet, an outlet and within it a total of 255 traps. We define the inlet value (velocity = 1.17 m / s) from the flow rate (3.6 ml / h) and the channel geometry and the outlet value (pressure = 0 Pa), using Navier-stokes equation by the finite element method. Subsequently, the real experiment was carried out, which lasted 48 hours. It was observed that where there was greater cell retention, which is a higher trapping rate, was in the traps that are located on the edges of the channel, but this occurs only at the beginning of the channel, because when observing after column 8 the speed of the flow becomes more homogeneous, that is, throughout the channel there is greater retention.

The convergence of the solutions was tested by evaluating the speed changes in the separation of each column, taking into account the scale of the mesh (normal). After knowing the behavior of the velocity in the whole channel, it was found for each individual trap, only in the first column, in order to know the pressure profile, obtaining that where the mother cell is trapped is where the flow is very weak, causing the pressure gradient to generate a negative force (opposite to flow). Daughter cells detach due to a positive force (in the same direction of flow). Finally, from the results we can see that the pressure profile for each trap makes it possible to trap the mother cell and separate the daughter cell. These results can be verified with data from the real experiment, where the same behavior of the simulation is observed.

Keywords: Aging, Hydrodynamic trapping, Navier-stokes equation.

Introduction

Saccharomyces cerevisiae yeast is a model organism used for the study of aging in eukaryotic cells (Mortimer and Johnston, 1959; Müller et al., 1980; Kaeberlein et al., 2005; Steinkraus et al., 2008; Breitenbach et al., 2012; Longo et

al., 1996; Fabrizio and Longo). The traditional method with which aging is studied uses micromanipulators, which makes it difficult, because great precision is required in the construction of these devices, causing it to consume a lot of time and at the same time many resources. The use of micromanipulators and the mechanical pressure they exert on the cell can affect its physiology (growth rate, genetic expression, aging, etc.), which would bias the results of these experiments. As an alternative, we propose a microfluidic platform that does not use mechanical but hydrodynamic trapping. This device must achieve the objective of trapping the mother cell, taking advantage of the slipstreaming effect, to let the daughter cells escape, requiring a very precise adjustment of the flows. To design this device, multiphysics simulations were carried out in order to test different parameters and select the optimal ones. In this work, we present the results of these simulations and their experimental application for the development of a platform that automates aging measurements in yeasts.

2. Mother Machine Design

The creation of the channel in the software is given by the measurements obtained from the Mother machine.

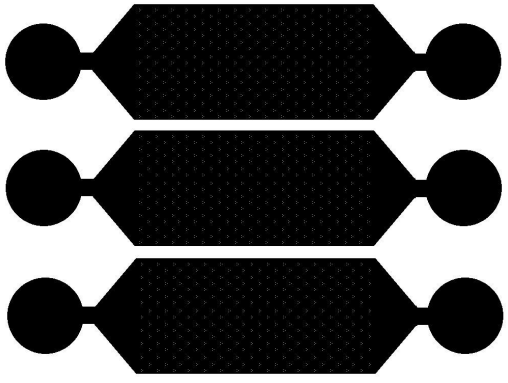

Figure 1: Design of the mother machine.

A channel consists of an inlet (left), an outlet (right) and within this a total of 255 traps, divided into 15 columns of 9 traps and 15 columns of 8 traps.

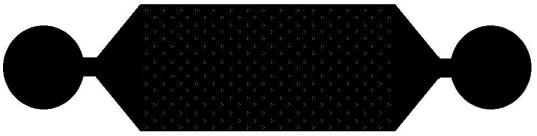

Figure 2: Design of a channel of the Mother machine.

Each trap is made up of three pillars which recreate the shape of a triangle.

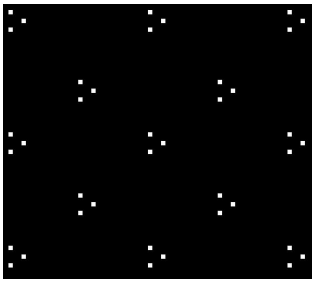

Figure 3: Shape of the traps.

2.1 Definition of parameters

Preliminary experiments were carried out where different variations were made regarding the shape of the traps and the behavior of the flow within the channel.

The shape of the traps, figure 3, is optimal since the three pillars seen as the vertices of a triangle, cause that when the flow passes through them, it does not cross them but surrounds them, causing it to gradually reduce the speed , generating the effect of slipstreaming due to the difference in flow velocity at the beginning of the trap and at the end of it, causing the mother cell to be trapped without being pressed.

The optimal flow quantity for our experiment was 3.6ml / h, the effect being much better and the syringe change is carried out every 15 hours, since if this is less there would be an agglomeration of

cells within the channel and if it is much greater than this, the syringe change would have to be done every 6 hours or less.

To obtain the initial velocity with which the flow enters the channel, the flow equation was taken into account.

$$\Phi = A * v \qquad (1)$$

Where Φ is the amount of flow, *A* is the area of the channel and *v* is the velocity.

The channel area is formed by a base that measures 170 μm and a height of 5 μm, where this is given by the approximate size of the cell.

By isolating the velocity from the flow equation (1), it was obtained that this initial value is 1.17 m / s, which is defined in the software as the inlet value and the outlet value would be the pressure where it is equal to 0 Pa, since it defines it.

2.2 Using COMSOL Multiphysics

According to the measurements taken in figure 2, in the paint software, knowing that a pixel is equivalent to a square with side 5 * 5 µm, the geometric construction of the Mother machine channel was carried out.

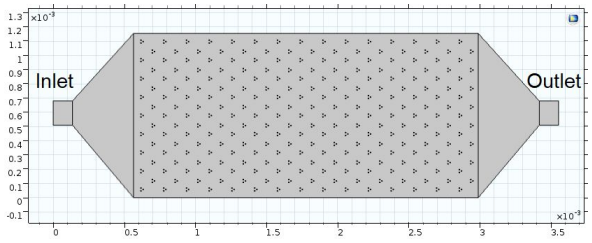

Figure 4: Geometry of the Mother machine channel.

In addition, a mesh adjustment was carried out, which was left in normal mode, to obtain greater precision in the data.

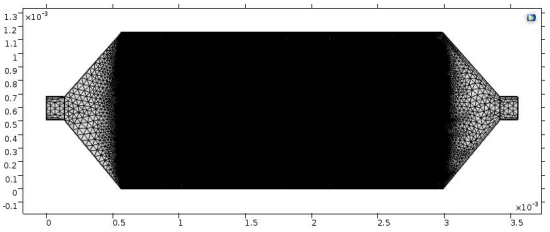

Figure 5: Normal mesh. Where it becomes thinner when there are traps present, while it is thicker where there are none.

3. Finite element method

The slipstreaming effect was modeled by numerically solving the Navier-stokes equations, by the finite element method in COMSOL Multiphysics. This being a numerical method which consists of the approximation of solutions to complex partial differential equations.

$$\rho(u \cdot \nabla)u = \nabla \cdot [-pI + \mu(\nabla u + (\nabla u)^T)] + F\rho\nabla \cdot (u) = 0 \qquad (2)$$

Where ρ is the density, u is the speed, μ is the viscosity, F is the strength of the body, ∇ is the gradient and p is the pressure

4. Analysis and results

First, the behavior of the flow within the channel is modeled, obtaining boundary conditions and the average velocity for each trap there.

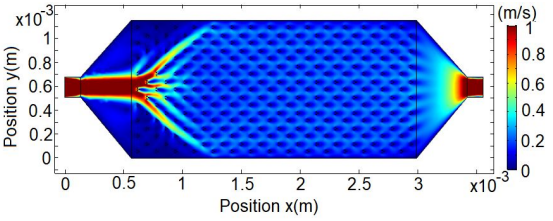

Figure 6: Channel velocity profile.

After obtaining the velocity profile throughout the channel, point-to-point data was taken after each column from the top to the bottom and thus obtain the different average speeds, thus giving the behavior of this, where in figure 7 is you see that the flow is not stable in the first 8 columns, while after this it becomes more stable and homogeneous.

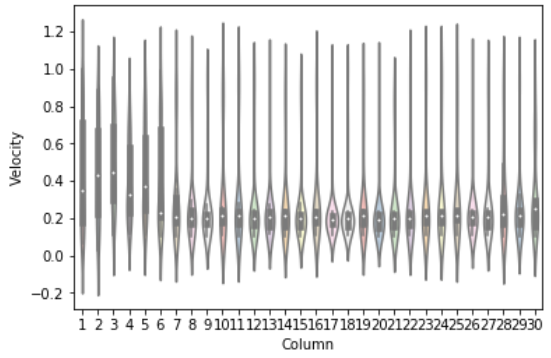

Figure 7: Columns and average channel velocity.

By obtaining the average velocity and the boundary conditions for each trap, the first 9 traps of the first column of the channel were modeled individually, this is done since the differences in sizes cause the program to crash. From there obtain for each one the velocity profile (figure 8) and the pressure profile (figure 9).

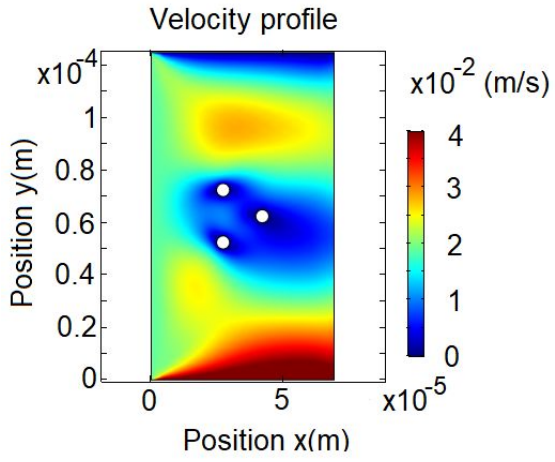

Figure 8: Velocity profile of a channel trap.

Figure 8 shows the velocity profile of a trap inside the channel, where a low velocity zone can be seen on the right (zone in blue).

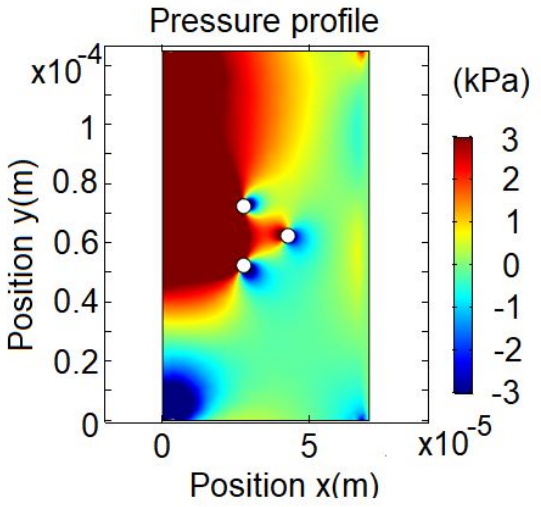

Figure 9: Pressure profile of a channel trap.

Figure 9 shows the pressure profile for a channel trap, where the pressure gradient can be observed on the sides of the pillars, this being negative, on the right (zone in blue). Then a dotted line was drawn in front of the trap, taking into account that each point represents a pressure data, resulting in figure 10.

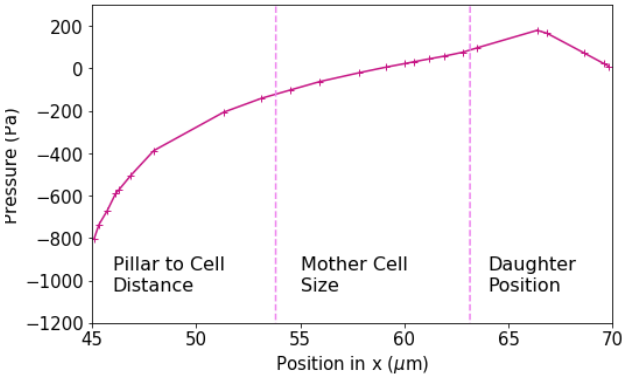

Figure 10: Pressure profile for a trap.

Figure 10 represents the typical pressure profile for a trap, since the mother cells are trapped by the negative force due to the pressure difference just after the last pillar where the flow velocity is very low and the daughters separate from according to a positive force, since they

are not in the same area of the mother cell and are carried away by the flow. The section on the left represents the distance from the end of the trap to the cell, the middle section shows the capture area and approximate size of the mother cell, and the section on the right shows the mother cells with their cells. daughters and then beak shows how they separate.

5. Real experiment

The real experiment was carried out, taking into account the different parameters used in the simulation in order to make a comparison with the data obtained in the previous section.

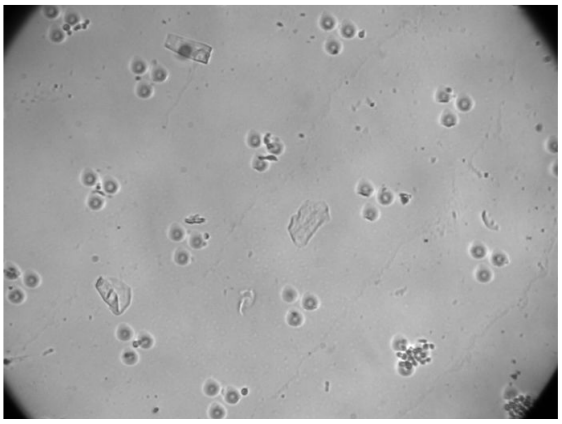

Figure 11: Capture of a section of the traps.

Figure 11 shows the capture of a section of the traps found in a channel seen by the microscope, these captures were made every half hour in different sections of the channel, this in order to obtain different images for 48 hours and continue the behavior of stem cells in traps.

To do the proper analysis of the images obtained from the real experiment, the fiji software was used, since it allows the visualization and measurement of each image. According to the above, it was corroborated in figure 10, the approximation of the distance from the trap to the cell, the size of the cell and the moment when it has a daughter and he separates.

Each selected trapped cell is monitored, that is, only one trapped cell remains and not an agglomeration of them, marking the survival time in each interval and the reproduction count.

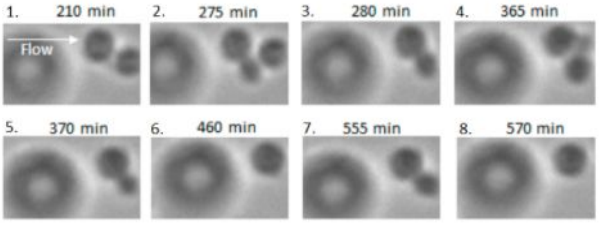

Figure 12: RLS measurements at different time intervals.

Figure 12 shows different photographs of a stem cell trapped in various time intervals, the largest circle being the pillar of the trap, opposite this is the stem cell and each small circle is a daughter cell. In (1) you see the mother cell with a daughter cell, in (2) the mother cell with its second daughter cell, in (3) the first daughter separates while the second continues to grow, in (4) the mother cell is found with the third daughter cell, in (5) the second daughter cell separates and the third daughter cell is growing, in (6) the third daughter cell separates, in (7) the mother cell with its fourth daughter cell and in (8) the fourth daughter cell separates.

6. Discussion

Taking into account that within the channel the behavior of the flow was not totally homogeneous, an improvement was made to the channel where at the beginning of it a branching was made in figure 13.

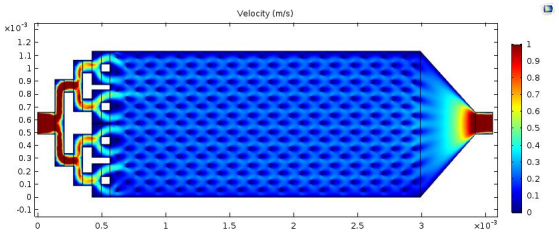

Figure 13: Speed profile of the branched channel.

After obtaining the velocity profile throughout the channel, point-to-point data was taken after each column from the top to the bottom and thus obtain the different average speeds, thus giving the behavior of this, where in figure 14 it is shown see that the flow is more stable and homogeneous from the first column.

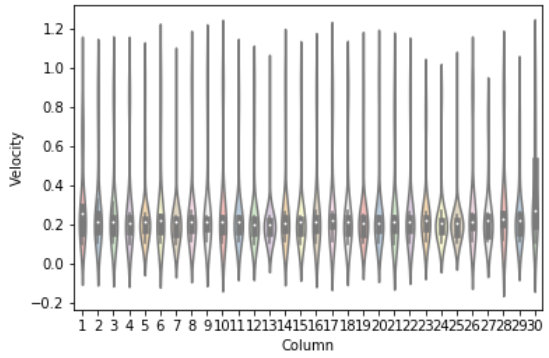

Figure 14: Columns vs average speed of the branched channel.

12 optimization rounds were carried out in which the first is figure 13, where the variation that was made was in the part of the branch, since as can be seen in the corners there is a blue part in which at the time of making In the actual experiment, there could be an agglomeration of cells causing the channel to collapse or the information to make no sense, for this reason, different modifications were made to the channel, finally positioning the branch at the beginning of the channel (figure 15), achieving that the flow already inside it was homogeneous from the beginning of it.

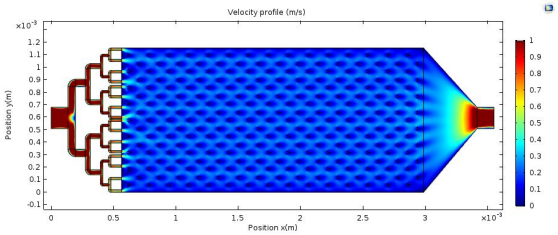

Figure 15: Speed profile of the branched channel, final version # 12.

7. Conclusions

- The flow profile in the complete channel was obtained, which was very useful since it allowed obtaining the average velocity data for a more detailed analysis in each individual trap.
- It was observed that in the traps there is a pressure profile that allows to trap the mother cell and separate the daughter cell, taking advantage of its shape and its arrangement in the channel, in addition to the slipstreaming effect generated by the direction of flow and the same.
- The results obtained from the simulation conform to the experiment carried out. Evidence of the effectiveness and usefulness of this type of simulation, since they allow anticipating the behavior of the real experiment, being safer the course that it will take, in addition to avoiding problems in its development and unnecessary losses of the budget.

8. Bibliography

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