# APP N02-5: FINAL MICRO WIP

# THE EFFECT OF SALINITY ON CELL ADHESION

Engineering 1282H

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## 1. Abstract:

In the world of microfluidics, attention is being shifted to the development of biofilms and their presence in medicinal applications. Since bacteria colonies can form biofilms in virtually any type of human serum, investigating methods to disrupt their growth is of great importance to develop superior medical technologies. Our experiment involved designing and building a PDMS microchannel, incubating a yeast biofilm inside, and testing gravity-assisted saline flow through the channel. The salt concentrations of each saline solution was varied between tests to determine whether salt concentration can affect biofilm shearing during flushes. Finally, statistical analysis was used to analyze the results, with a null hypothesis of no change in yeast cell microchannel adhesion at different salt concentrations. We were not able to complete this lab experiment, so results remain tentative. More testing of biofilm adhesion to channel walls should be studied with saline as well as other compounds to determine more of their characteristics.

## 2. Introduction

As innovation and new technologies further shrink existing tools, more testing is required to validate its potential uses. In the world of microfluidics, each application must be carefully evaluated before gaining status as a useful substitute for equivalent experiments on a larger scale. Microfluidics requires a deep understanding of the physics of fluids on a small scale. This field is garnering interest because its small size makes it easier to investigate the behaviors of cells. Microfluidics have been especially useful in studying the nervous system of bacteria such as C.Elegans, otherwise known as roundworm [2]. It is easier to consolidate small bacteria studied on a small scale, making microfluidics work especially useful.

More and more, increased attention is being given to the study of biofilms, thin layers of proteins and lipids created by microbes that suspend and protect their network of cells [6]. As bacteria have a particularly superior ability to form biofilms in developed colonies, study of them is useful for determining key characteristics of the phenomenon. These films have the potential to form in medical devices, *in vivo* in live animals, and on virtually any surface that adequately protects the bacteria from environmental stress. Additionally, Biofilms are used to understand infectious diseases and how they can be controlled. By investigating how the growth of biofilms and cell adhesion can be inhibited, many infections can be controlled. Much the work done in this experiment comes from previous work done by Mercier-Bonin. Her work outlines the use of microfluidics in studying biofilm formation [9].

Previously, biofilms had been studied as natural results of the presence of bacteria within serum or another habitable medium, with resistance to environmental stressors determined partly by the combination of symbiotic culture [10]. These biofilms thus have demonstrated the ability to form inside medical devices as their implementation in human applications leads to areas exposed to human sera without adequate cleaning. Because biofilms can adhere to even plastic and acrylic surfaces, cell adhesion is an important component to study.

With the application of bacterial biofilm formation in medical devices a facet of important medical experimentation, our research focused on the various effects of biofilm formation and survival. Our experiment revolved around using a PDMS-based microchip and microfluidics processes to test yeast cell adhesion on microchannel walls. Our alternative hypothesis was that salinity did affect the shearing of yeast cells within the microchannel walls, and higher levels of salinity could lead to increased destruction of a yeast cell biofilm. Salinity has important applications in the real-world because saline solutions are often used to clean medical equipment.

## 3. Objectives:

This research experiment investigated how salinity affects cell adhesion of yeast cells. The concentration of salt varies naturally among different environments, such as in oceans, rivers and lakes, and on land. Observing how cell adhesion is affected by different concentrations of salt solutions will give insight into how cells react in these various environments. Specifically, it will be clear how higher salt concentrations allow for cell adhesion. There is an important distinction to be made here, as this study does not investigate how cells will grow in environments of higher salinity, but rather how cell adhesion is affected when flushed with a more saline solution. The hypothesis is that a higher salt concentration will reduce how well the cells can adhere to each other and their surfaces. This hypothesis was made based on a study that tested how charge regulation affected cell adhesion [5]. This study showed that an increase in salt also increased the repulsion between the cells. This statement was supported by several other studies as well [4,7]. Because the salt would serve as a charged component in the channel, the yeast cells may be more attracted to the dissociated ions that are now present.

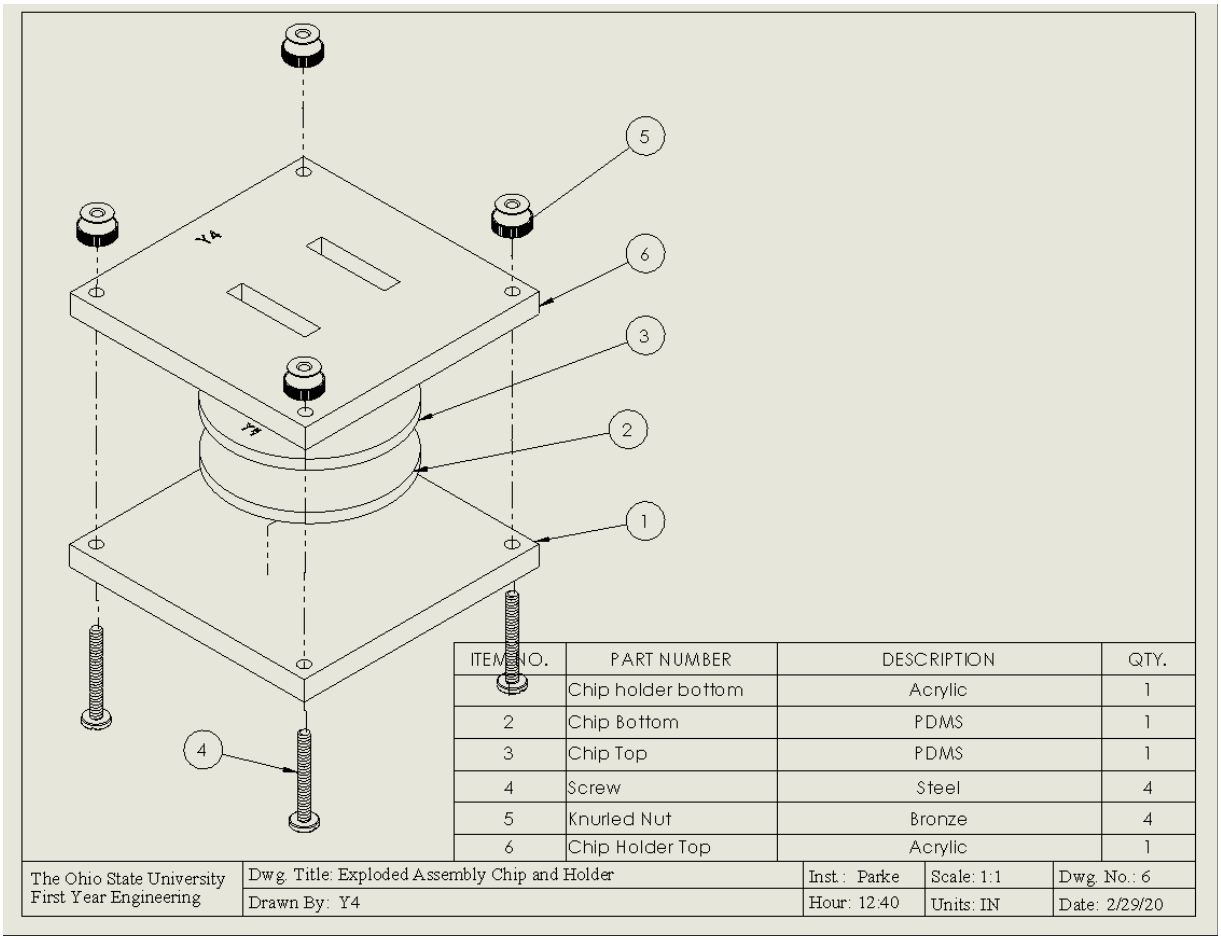
The results of this experiment will be useful in determining if adjustments should be made when growing yeast in environments with high salinity. These results may also allow for reasonable assumptions to be made when studying biofilm formation. This study should provide insight into how biofilm formation can be controlled, and how best to suppress it in medical applications. Writ large, the results from this work may be applied to a large field of microfluidics work, may help to further general knowledge of bacterial behavior at the microscale, and may help test new technologies to inhibit biofilm formation.

## 4. Methods:

Yeast cells were used in their experiment because of their easy access and simple mechanism. The adhesion of these cells relied heavily upon shear stress models. Shear stress is the component of force that is parallel to the surface. By initiating a flush, shear stress is generated and aids in removing unadhered cells.

### a. Construction of Chip:

The chip that was used in this experiment was first designed in SolidWorks, a Computer Aided Design (CAD) software to design devices. The chip itself had 4 small channels that 24.23 mm in length, 0.25 mm in depth, and 0.18 mm in width. These chips were cured and created in PDMS. Additionally, a chip holder top and bottom were also created to hold the chip while the washes were performed. The chip holder top and bottom were made from acrylic so that they would be more stable. The channels in the chip were designed to allow for laminar flow without overwhelming the yeast cells with too much fluid. The channels also had inlet and outlet ports on each side. The outlet ports were designed to be smaller than the inlet ports, a feature that made the channel more conducive to laminar flow. Laminar flow was important for this experiment because it ensured that shear stress was appropriate. When flow is laminar, the shear stress is maximized at the walls of the channel, which allows for the removal of yeast cells that have not adhered. The laminar flow was ensured through SolidWorks. Using the Computational Fluid Dynamics component of this software, the channel was tested to see how a Newtonian Fluid would flow through it. The chip holder top and bottom have openings big enough to insert water into the entry port and absorb the water from the exit port. The openings are 25.40 mm in length and 5.08 mm in width. They both had four holes at the corners for screws to hold the entire chip together. The exploded assembly of the chip is shown below.




| ITEM NO. | PART NUMBER | DESCRIPTION | QTY. |
|---|---|---|---|
| | Chip holder bottom | Acrylic | 1 |
| 2 | Chip Bottom | PDMS | 1 |
| 3 | Chip Top | PDMS | 1 |
| 4 | Screw | Steel | 4 |
| 5 | Knurled Nut | Bronze | 4 |
| 6 | Chip Holder Top | Acrylic | 1 |

| | | | | |
|---|---|---|---|---|
| The Ohio State University<br>First Year Engineering | Dwg. Title: Exploded Assembly Chip and Holder | Inst.: Parke | Scale: 1:1 | Dwg. No.: 6 |
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Figure 1: SOLIDWORKS Drawings for the microchip assembly. Screws, bolts, and all chip parts are included in this assembly.

## b. Experimental Design:

A tower was first set up to create gravity-assisted water flow. The tower was composed of a metal base and metal rod that extended several feet upward. A tube holding device and a ruler were connected to the metal pole. A syringe was then attached to the metal pole by moving the plastic holder up or down on the pole and stopping at the desired height. A tube connected at the bottom of the syringe and ran down to the bottom of the tower, where it was closed off with a plastic clamp. The syringe was filled with water, and the other end of its tube was connected to a microchip held on the lab table by one team member. One of the team members held the end of the tube, and connected it to the inlet port on the microchip. A second tube was connected to the chip's outlet port and held in place by another team member.

The first experiment began at 25cm height. At each "height" increase, the top of the tube was raised 10cm from its earlier position (the original position being 25cm). There were three

trials, each with three tests at one height. Heights were varied in this experiment to gather more data for increased fluid velocities.

In the first few runs, only pure water was flushed at the different heights so as to determine optimal conditions for maximum and minimum biofilm shearing. These test results are described more thoroughly in section 5. After these test runs, however, salt content of the saline solution was varied and tested at three different heights.

Three concentrations of NaCl treatment that were tested were 25%, 50%, and 75% w/w, with water as a control. Pressure washes were conducted at the same height for each solution to prevent height becoming a confounding variable in the experiment. The yeast solution was prepared by mixing 2.0 grams of yeast with 10.0 mL of warm water in a beaker and incubating for 15 minutes. Once the yeast had incubated, it was injected into the chip and left to incubate for 20 minutes. Afterward, a low-pressure wash was conducted to remove any nonadhered yeast cells. This low pressure wash consisted of using the same me The amount of yeast that remained after the low-pressure wash was considered the initial amount.

Each trial consisted of a "low pressure wash", a rinse designed to remove excess floating yeast cells that had not bound to a biofilm during the incubation time. The trial then consisted of three "tests", each involving 60 seconds of gravity-assisted water flow from the tube into the chip. A team member assisted in constantly adding new water to the top of the test tube, ensuring a consistent water pressure going into the chip.

After each test, the chip was observed under a microscope for presence of yeast cells. The data point was accepted if at least 50% of the original cells were sheared. All data points were recorded in a lab notebook. The projected schedule is to collect one viable data point every day until five points have been collected for the control and two at each salt concentration.

### c. Statistical Analysis:

Once all data points have been collected, a one-sided t-test will be conducted at a significance level of 0.05. The t-test results will help provide more insight as to the affects of salinity on yeast cell adhesion, taken as the chance that the difference between our saline and non-saline test results could have happened at random.

## 5. Prior Results:

Leading up to this study, a series of other experiments and simulations were conducted. A Computational Fluid Dynamics (CFD) simulation was run in SolidWorks. Running CFD on the designed channel showed results that aligned with expectations. The velocity and shear stress contours were similar to ones from previously designed channels that were proven to have laminar flow. Shear stress and velocity increased as the pressure increased, a desired result. The pressure contours varied slightly as ours dispersed in a rectangular pattern instead of a circular one. Finally, the transition to turbulent flow happened at a Reynold's number of 2000, which is unlikely to occur in our channel given its smaller Reynold's number.

As aforementioned, there was a set of yeast trials that were conducted in order to fully calibrate flow. In this experiment, several pressure washes were performed at different heights, specifically 25,30,35 cm. This showed a very weak relationship between height and flow rate. There was one outlier that arose from improper use of the equipment. Overall, the shear stress and velocities were similar at these heights which led to the decision of using these heights in this experiment. In another iteration of this experiment, threshold shear stresses among different heights were similarly around 150-160. The threshold shear stress that the group calculated using height was 153.05 H, dyne/cm^2. Using flow rate, the group calculated the threshold shear stress 174.45 Q, dyne/cm^2.

## 6. Anticipated Results/Discussion:

The alternative hypothesis of this experiment is that yeast cells will shear more often as the flush solution's salt content increases. Prior studies have indicated that high salt concentrations could negatively affect the bacteria's adhesion capabilities [5] and that cell adhesion may be enhanced in solutions with very low salt concentrations [7], giving credibility to an anticipated decrease in cell adhesion when flushed with saline of higher salt content.

The study mentioned in the Objectives section (section 3) concluded that salt increased repulsion between the cells, making their increased affinity for adhesion to the microchannel walls quite plausible [4]. However, this study also considered the charge of the cells and the substrate that it would attach to; neither of these are a factor in our experiment, so results are not exactly comparable.

Another study tested how the formation of a biofilm would be affected by shear stress and salinity showed that low concentrations of the salt, 5 g/L, enhanced the formation of the biofilm. At higher salt concentrations, specifically 15 g/L, the biofilm did not form as well [5]. This aspect will also be considered in the proposed experiment as various salt concentrations will be tested. If the cells are more attracted to the channel walls at lower salt concentrations, then it is expected that more yeast cells will be observed in the channel after the wash with the lowest concentration. However, at the other two concentrations that are higher, the yeast cells may be washed out more easily, leaving fewer yeast cells to be observed in the channel. At this point, the effect of high concentrations of NaCl may be less associated with cell adhesion and more with cell deterioration. A study done in the 1960's suggested that the enzymatic composition of the cell would be affected by the presence of NaCl. This study used the tumorous HeLa cells, so their composition and behavior were likely different from the yeast cells that will be used in this experiment [3]. Further research comparing the structure of yeast cells and HeLa cells showed that yeast cells have a thicker cell wall than the HeLa cells do [1]. This implies that the results of this experiment will not be as drastic as the one involving HeLa cells. Overall, it is expected to see less yeast cells after the wash with the high NaCl concentration.

In another study, biofilms were treated with$CaCl_2$ and NaCl, then compared the two salt solutions on its development. They found that calcium chloride had a stronger effect on destroying the biofilm [7]. They hypothesized that the formation of biofilm increased in the

presence of salt since electrostatic repulsion forces were reduced. This means that the cells were more attracted to each other and therefore formed the biofilm. These results are applicable to our study because the yeast cells may adhere to each other and collectively be washed out. This supports the hypothesis that the higher salt concentrations will reduce the adhesion of the cells.

The results of this experiment will provide more information on both cell adhesion and yeast cells. The consensus of the current studies is that the presence of salt can enhance adhesion but only in low concentrations. There is no concrete theory that explains this phenomenon, so the proposed study may give more information towards finding the answer. Additionally, yeast cells are used in many different experiments. They have a simple mechanism which makes it easy to use but seeing how salt affects their behavior may help in other experiments where yeast cells are used. With the results of this study, other experimenters can decide how to construct the environment where the yeast cells are incubated. Finally, the results of this experiment can also be broadly applied to other types of bacteria or cell types. This will further the understanding of cell growth and adhesion in general.

## 7. Conclusion:

This experiment would show how cell adhesion and ultimately biofilm formation is impacted by salinity. The designed chip shows that microfluidics can be used to investigate properties of cells. Shear stress was very important to the proper functioning of the experiment, so that unadhered yeast cells will be removed. A one-tailed t-test will be run on the results to determine if they are statistically significant. If the p-value is less than the significance level of 0.05, then the null hypothesis will be rejected. The results will have many public health and medicinal applications because it will provide insight into how biofilm formation can be controlled.

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