Bio Roll Up: Maintaining Uniformity and Implementing Rinse Step

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I. Project Introduction

The goal of Bio Roll-Up is to determine how self-assembly can influence the differentiation of stem cells. A Bio Roll-Up is a patterned layer stack on a silicon wafer that self-assembles at low temperatures. The rectangular stack itself consists of two hydrogel biolayers that have different crosslinking concentrations. The bottom biolayer has a higher concentration than the top biolayer, allowing the stack to roll into a tube. The self-assembly is triggered by temperature due to what serves as the wafer-adhesive layer or release layer for the biolayers. This release layer consists of a thermally responsive polymer that dissolves in low temperatures. In the future, cells will be seeded on top of these biolayers, allowing the hydrogel stack to serve as a scaffold. Before cell seeding and during cell proliferation and attachment, the wafer will be immersed in phosphate buffer saline (PBS) at 37ºC. Ultimately, the stacks will be immersed in 7ºC PBS solution, and form tubes resembling salivary glands.

In the fabrication of the Bio Roll-Ups, the wafer is exposed to UV light in order to allow the biolayers to crosslink and become insoluble. A mask is used to create a check-board pattern, with soluble and insoluble squares. When the wafer is submerged in the development solution, the soluble squares, those covered by the mask when exposed in UV light, are dissolved. When the wafer is dried however, using a hot plate and heat gun, the solvent is evaporated, but the solute, the biolayers, are left on the silicon wafer. This affects data collection as it prevents accurate measurements for thickness of the biolayers. Furthermore, cell proliferation and seeding can be affected by nonuniform stacks. A rinse step can be implemented to dilute the solution, allowing the surface of the wafer to have a more uniform surface.

II. Materials

The Bio Roll-Up project utilizes recycled silicon wafers to fabricate the hydrogel scaffolds. The release layer formulation is calculated based on the desired molecular weight of 5%. The polymer, PNIPAM, is used due to its biomedically advantageous thermal responsive qualities. PNIPAM dissolves around a temperature of 37ºC, the temperature of a human body, and remains insoluble above those temperatures. The biolayer polymer consists of a copolymer of 2-Hydroxyethyl Methacrylate (HEMA) and Acrylic Acid (AA). To chemically engineer the hydrogels to self-assemble, two formulations with varying amounts of crosslinkers are used. Biolayer one consists of 10% crosslinker and biolayer 2 consists of 20% crosslinker allowing biolayer one to swell to a greater degree triggering the rolling motion of the scaffold.

The formulations of the release layer and hydrogel scaffold layers used for the main experimentation have been established as the prime formulations at that time. Alongside and just prior to this research, improved formulations for the release layer and "bio-layers" or hydrogel scaffolds were being developed. Formulations for the release layer were used mainly to visibly observe results of both the rinse step's performance and dissolution of the release layer, but this data was not used to create graphs.

In order to deposit and bake the release layer and biolayers, a spin coater and two hotplates set to 90ºC and 200ºC were used. A UV light exposure tool and 35% Methanol solution in a water bath is used to allow the formulations to crosslink and develop. For the rinse step both spray bottles and solvents in pyrex bowls were used. The solvents were isopropanol, methanol,

DI water, and hexane. Finally, A heat gun was used to dry the wafer. Both the profilometer and ellipsometer were used for data analysis. After analysis, to test the self-assembly of the Bio Roll-Ups, phosphate-buffered saline (PBS) is used.

III. Procedures

A. Using Profilometry to Measure Thickness Throughout the Wafer

In order to first observe the degradation of and depositing of the release layer precipitate on the rectangles for the established formulation and fabrication process, a profilometer was used to measure the thickness of the rectangles throughout the wafer. A wafer was fabricated as normal using the best formulations of biolayer and release layer at that time. A rectangle on the edge of the wafer, the middle of the wafer, a partially degraded rectangle, and a deeply rectangle were measured. The highest average thickness were recorded as well as the lowest average thickness were recorded and the difference between the two peaks were calculated.

B. Original Procedure

The original fabrication procedure does not involve any rinse step. The fabrication process begins with the release layer being deposited using a spin coater at 1,000 rotations per minute for 45 seconds and baked at 200ºC. The same process is followed for the biolayer, but the hotplate is set to 90ºC. After cooling, the wafer is exposed to UV light for 800 seconds, and submerged in 35% methanol solution for 1 minute. The wafer is transferred to a hot plate at 50ºC and dried at an angle using a heat gun.

C. Rinse Step Using Spray Bottle and Testing Procedure

The first solution to the problem of the lack of uniformity in the Bio Roll-Up was to use a spray bottle filled with 4 different solvents at room temperature for a rinse step. After depositing the formulations, the wafer was exposed to UV light for 800 seconds then cleaved into four parts. Three out of the four parts were developed for 60 seconds and held in a tilted manner while being sprayed with a solvent: DI Water, Hexane, or Isopropanol. One part was fabricated normally by being submerged in 35% methanol solution for 60 seconds. All were dried using a heat gun and hotplate. This process was repeated with each solvent heated to 37ºC and then sprayed onto the wafer. A third experiment was conducted where the Bio Roll-Ups were fabricated without the release layer.

D. Rinse Step using bowl of DI water and Testing Procedure

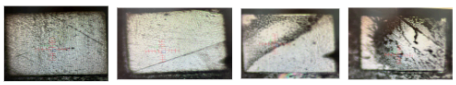
In place of the spray bottle, one-half of the wafer was submerged into a bowl of DI water at 37ºC for 30 seconds. During the submerging, the bowl of DI water was slightly swirled to clear away any fog from the precipitate. The other half of the wafer was fabricated as usual, and both were dried using a hot plate and heat gun. After testing, a new experiment was conducted with the wafer being cleaved into two, then one half cleaved into two again to form three pieces. One of the smaller pieces was submerged into DI water for 15-20 seconds before developing for 60 seconds in 35% methanol solution and then submerged again in DI water before drying. The second smaller piece was submerged after development, and followed the same procedure as above.

The regular testing procedure involved using a release layer was used and tested at 37ºC and 7ºC. For testing in 37ºC, the pyrex bowl is filled to about half and placed in a water bath at 37ºC. Each of the four or three wafer parts are cleaved again into two pieces. The number of rectangles on each piece are counted before being submerged in the 10% PBS solution. The number of roles that were self-assembled were counted over an interval of 60 minutes. This process was repeated with the second piece that was submerged into 7ºC. The underlayer testing procedure differed in that the wafer was not cleaved a second time and instead was submerged in room temperature 10% PBS solution. Self assembly was recorded until full self assembly of the rectangles.

IV. Data Analysis

Throughout the process, pictures were taken to document the appearance of the wafer at each step to visually observe the uniformity of the wafers. Pictures of individual rectangles were taken with a profilometer. To measure thickness and rectangle uniformity, the profilometer graphs were used. The data from each experiment were graphed based on temperature and compared each solvent with each other.

V. Results and Discussion

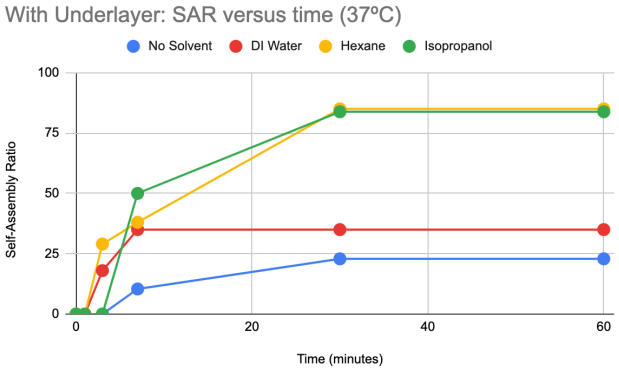


Identification	Edge of Wafer	Middle of Wafer	Partially Dirty	Mostly Dirty
Highest Average Thickness (micrometers)	2.39	2.11	2.52	2.72
Lowest Average Thickness (micrometers)	1.96	1.80	1.46	0.52
Difference (micrometers)	0.43	0.31	1.06	2.2

In the initial data collection of the rectangles made with the original formulation and fabrication process, the differences between the thickest part of the wafer and thinnest part of the wafer was measured to understand the lack of uniformity. The edge of the wafer appeared

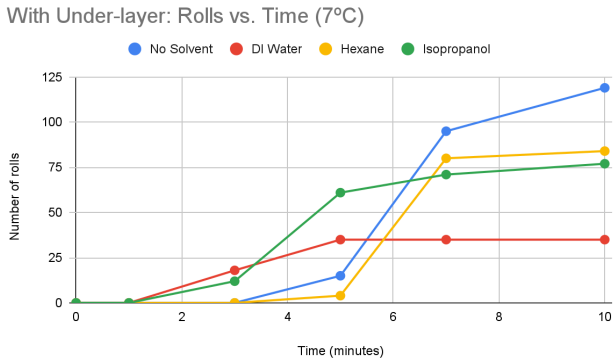
was measured to have a greater difference, 0.43 µm, than the middle of the wafer 0.31 µm. Both these numbers were in great contrast with the rectangles that seemed very degraded. The partially degraded rectangle had a difference of 2.2 µm. Overall, the highest average thickness of all the rectangles stayed consistent with a highest of a 0.61µm difference between the middle of wafer and extremely degraded.

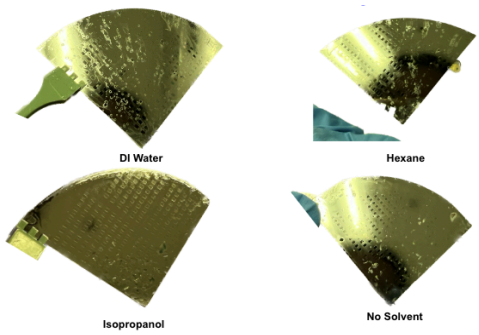
The self-assembly test with the current formulations showed greater self-assembly in 7ºC than 37ºC. However, in transferring the Bio Roll-Ups to 7ºC after being submerged in 37ºC 10% PBS solution no self assembly was observed. This was done to observe the behavior of the rectangles, as in the future the rectangles must be able to self-assemble with that transfer, therefore the lack of self-assembly may indicate further research for the release layer formulation. For the following experiments, self-assembly was used to determine which rinse step will both not affect self-assembly negatively and produce a more uniform wafer and rectangles.



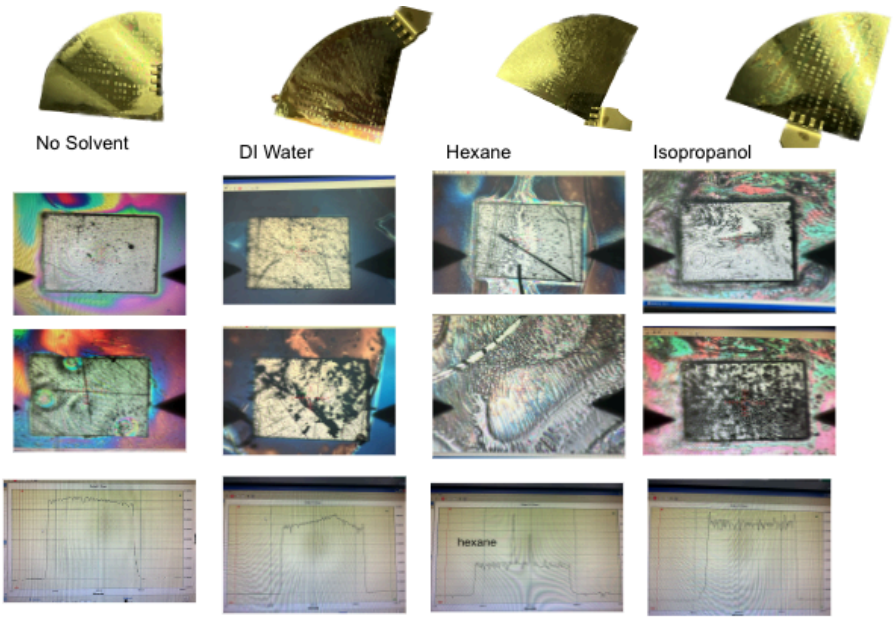
For the first spray bottle experiment with room temperature solvent, some rectangles rolled up when exposed to the solvent, most likely because the release layer dissolved in response to the colder temperature. However, all three solvents were still tested. At 37ºC, where the rectangles should not roll up, Isopropanol and Hexane yielded the most rolls. DI water and no solvent produced similar results, however, the no solvent yielded the fewest rolls and therefore had the best result. For the

experiment in 7ºC, no solvent once again had the best result, yielding the most rolls. Hexane and

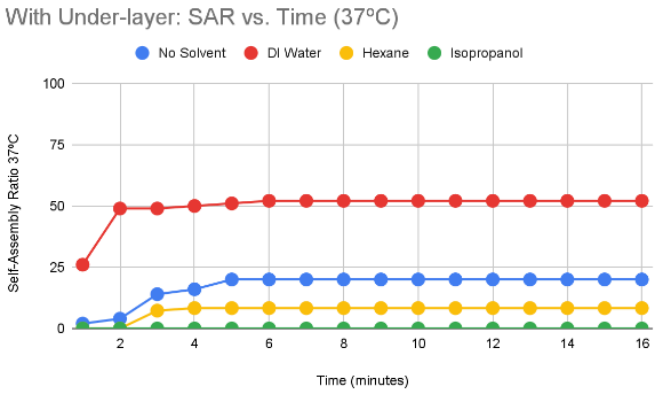




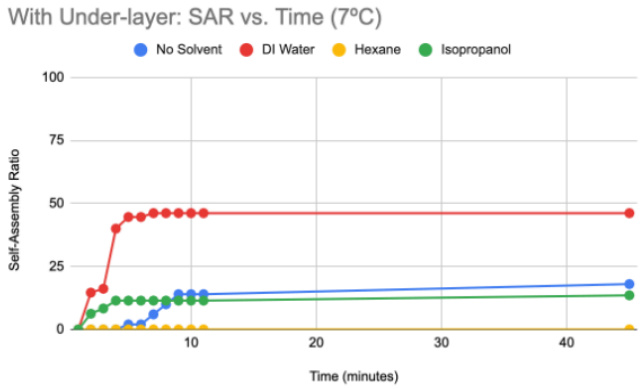
isopropanol came in second and third and DI water came in fourth, yielding about the same number of rolls compared to its performance in 37ºC. Furthermore, no solvent also looked more uniform than the other pieces exposed to the solvents.



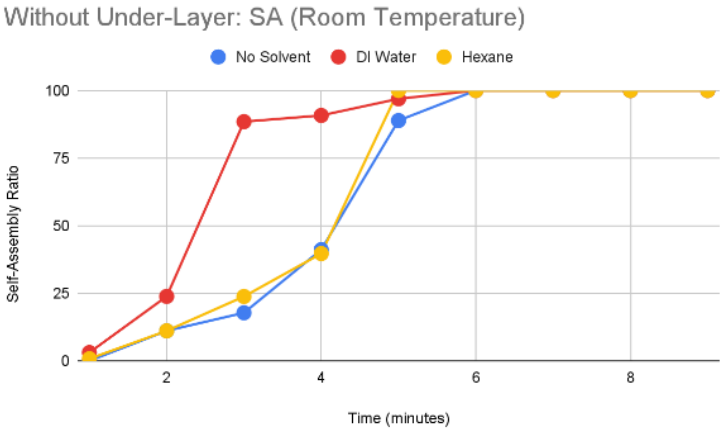
A second experiment was conducted with the spray bottle, this time with each solvent heated to 37ºC using a water bath. This time, there were few to no rolls after being exposed to the solvents and appearances in general differed. DI water seemed to have completely dissolved the release layer surrounding the biolayers, giving it a smoother appearance. A cloudiness formed on the



piece with no solvent and hexane, and the piece exposed to isopropanol seemed to have a shiny appearance. When measuring the average rectangle of each solvent, no solvent and DI water were most similar in uniformity and hexane and isopropanol were most degraded. Another self assembly test was conducted and at 37ºC and 7ºC, DI water had the most rolls. Interestingly, no solvent came in second place for both 37ºC and 7ºC. These results were the opposite of the prior experiment, but in each experiment self assembly at 37ºC was never zero and self-assembly at 7ºC was too low at around 50%.

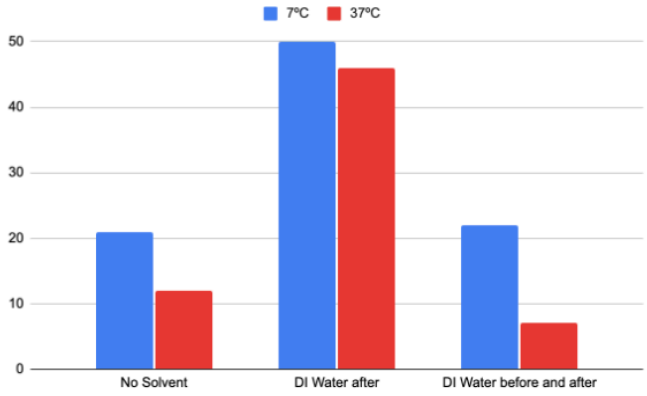


In order to reach full self-assembly, another wafer was fabricated without the release layer. Only two solvents were used this time: DI water and Hexane. DI water reached self-assembly first and no solvent was last. Hexane had the most degraded rectangles out of the three and was ranked last in appearance. No solvent looked the most uniform and DI water came in second.



Because DI water was yielding the most similar results as no solvent, a new experiment with only DI water as the solvent was conducted, where a bowl method was used instead. The rinse

step using a bowl of DI water yielded the most interesting results. This was mostly likely due to the release layer formulation, as a different formulation was used. The first experiment comparing the piece with DI water rinse after development and the piece with no solvent



produced no self-assembled rolls at all at both 37ºC and 7ºC. A second experiment was conducted, with three pieces of wafer. One submerged in DI water before and after development, one submerged only after, and one with no solvent. The piece submerged DI water before and after development produced the best results for 37ºC. The piece submerged in DI water at 37ºC after yielding the most rolls during 7ºC. Ultimately, the piece submerged in DI water before and after had the best results, with slightly more rolls in 7ºC than no solvent as well. The piece submerged in DI water may have had the release layer dissolved much more, allowing for more self-assembly.

VI. Future Work and Conclusions

In conclusion, a rinse step which combats degradation and depositing of the precipitate is necessary to allow for measurement of the Bio Roll-Ups. Furthermore, a lack of uniformity in the rectangle can affect cell proliferation and seeding. For the majority of the experiments, no solvent seemed to yield the best results. However, the last experiment showed how the DI water rinse before and after yielded the best results. This may be due to a more uniform surface and the release layer's response to the DI water. The greater amount of self-assembly for the rinse step

with DI water indicates that the release layer perhaps needs to be able to dissolve a greater amount to produce more rolls as very few rolls were produced for the piece with no solvent.

For future work, a better rinse step may be to rinse the wafer with 35% methanol solution after development at 37ºC. Furthermore, different amounts of the release layer and sublayers should be experimented with to better understand thickness of these layers in relation to self-assembly. A formulation for the release layer that most effectively enhances the polymer's thermoresponsive qualities can potentially improve the transfer between 37ºC and 7ºC.