Development of a cell culture platform in PDMS
Microfluidic systems for in vitro production of platelets

Nicki Nordh
Abstract

Development of a cell culture platform in PDMS

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To be able to effectively study blood platelets in different environments a development of an in vitro model of a microfluidic system for platelet production was started. The purpose of this thesis was to fabricate systems and then characterize them and visualize the flow. The system consists of two channels, one in the middle and the other one enclosing it. They are connected through pores where Megakaryocytes can protrude through and produce platelets. The designs were produced in PDMS. This was done by first transfer the designs as structures onto a silicon wafer through UV lithography. The wafer served as a mould for casting PDMS that later was bonded to glass. The systems were then studied with three different methods. Computer simulations, flow tests and ultimately tests with cells. From the results new designs were made and fabricated. The new designs were then tested the same ways as the first ones. The systems can most probably produce platelets with some optimisation of the test parameters. No definite results were gathered to prove platelet production. Different flow speeds were tested and the flow profile at different flow rates was visualised. The full capability of the new designs could not be fully studied due to unforeseen debris of PDMS clogging the channels. A few things need to be done to achieve better results and establish for sure if this method of producing platelets is possible. This thesis is a good ground for future work to stand on.
Preface

Thesis background
This Master thesis was the last part of my master degree in chemical engineering. I have done this thesis at the department of Micro system technology at Ångstrom laboratory, UU Uppsala University. I sought Maria Tenje for her inspiring and interesting research and got this thesis offered to me. It was a work of collaboration between two groups at Uppsala University, Maria Tenje at MST and Johan Kreuger at medical cell biology (MBC). It was decided that Maria Tenje would be my supervisor, Johan Kreuger my secondary supervisor, Klas Hjort my subject reviewer and Erik Lewin my examiner of the thesis and presentation. This thesis was done between January and June 2015.

Acknowledgement
First of all I would thank my supervisor Maria Tenje for offering this thesis to me. She have during the process always been there offering support and feedback. By letting me work with this project I gained valuable experiences. She made me feel welcome in the research group and made this an enjoyable time for me.

I would like to thank Rodrigo Hernández for helping me with parts of the project and Frida Sjögren for always being there and discussing my ideas with me. With your help this work proceeded more smoothly than it would otherwise.

I would also like to thank my wife for the love and support she always give me. Without her I would not have been able to reach this far in life. Thank you!

“A well-spent day brings happy sleep.”
- Leonardo da Vinci
Sammanfattning

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Introduction
The field of medicine has grown in recent years [1]. New ways to help people in need have been discovered throughout the years. One of these ways is transfusion of living cells and tissues. Since this includes living things with a specific lifetime there is a constant need for donors to keep the storages from being empty [2]. Platelets, one of the blood components, has an important role in not only haemostasis and thrombosis. They are also important in the maintenance of the vascular integrity and the innate immune response [3]. Over the last decades there has been a rising demand for transfusions while the pool of donors has slightly decreased [4]. This means more of a shortage of platelets for the patients. Because of this an interest of developing an in vitro production of platelets has been created [4]. It would also be of great value to be able to produce platelets since they can be used in wound treatments [5].

There is a need to be able to perform clinical studies of platelets in different environments. And by producing an in vitro method of platelet production the access to platelets would be greater and be of great use to future research.

To be able to design a system with a purpose of producing platelets, an understanding of how the cells normally produce is needed. Hematopoietic stem cells (HSC) are the cells responsible for producing most cells in the bloodstream. To make platelets, first the HSC become a progenitor cell which in turn evolves into a megakaryocyte (MK). The MK can then produce platelets. Figure 1 illustrates this chain of changes. The process when the platelets are formed is quite special. In vivo the MKs are placed inside the bones but very close to the surface. By growing extensions out of the pores in the bone into the bloodstream called pro-platelets the platelets is then “shaved” off due to sheer stress from the passing blood [6]. An illustration of this can be shown in Figure 2. This means two different flows are needed in such a system, one for transporting megakaryocytes and one for applying shear stress and collection.

![Figure 1. Schematic figure showing the chain of changes a HSC undergo to produce platelets. Source: [4]](image)

When knowing this it is easier to come up with an idea of how to design a microfluidic system able to produce platelets. Earlier studies in this area [2, 7-9] show examples of ideas of possible designs to achieve this. Illustrations of two ideas for systems can be seen in Figure 3. Generally it is two ideas mentioned in earlier studies. One where there are two inlets and two outlets. The two channels meeting in the middle separated by a wall with holes. By varying the flow through one outlet (on the MKs side) the cells will be redirected through the wall. Since the holes are too small for the cell to
pass through, the MKs start to grow extensions to the other side and the other flow will apply shear stress and platelets will fall off [2]. The other idea differs mostly in that only one outlet exists. The flow carrying the MKs will also be used for applying pressure on the cells to help them through the small pores entering the second flow [7]. These ideas are shown in Figure 3.

Figure 2. An illustration that shows how a MK extends into the blood stream (pro-platelets) and the shear stress from the blood flow helps produce platelets. Source: [6]

Studies in this field have focused on achieving a system capable of producing platelets at the same rate as the human body. If such a system could be manufactured, the dependence of human donors would decrease. Different ideas have been discussed in effort to replicate the natural environment in the body where the production of platelets occurs. The key point for a functional system is the shear stress shaving off the platelets. In vivo the normal stress is approx. 120 mPa in the smaller blood vessels [2]. If as seen in the second design in Figure 3, two flows merge into one, one flow can be used for applying pressure on the MKs. By applying pressure on the megakaryocytes they are stimulated to produce platelets [7].

Figure 3. Two ideas for a system where MKs can produce platelets for harvesting. To the left the two flows are separated by a wall with holes. To the right the flow carrying the MKs merge with the other one. Source: [2, 7]
**Task**

The research about MKs and platelets require a microfluidic system to be able to replicate the production of platelets in the body. By cooperation between two research groups at Uppsala University, existing designs will be characterised. With the results given from the characterisation new suggestions of designs will be made. The flow will be visualized in existing and new designs.

The goal of this MSc thesis work is to manufacture different systems, both existing and new designs and to characterise them. The systems must be made so that megakaryocytes can be placed at pores and be able to produce platelets as described above and illustrated in Figure 2. Different flows will be visualized since understanding of the flows is necessary for better designs in the future. The information and system produced should help further research as tools to increase the understanding of how proteins work by testing modified platelets. The focus will be to manufacture the systems and get a flow profile of them. Then the process of platelet production will be visualized. If possible, the platelet production will be optimised and number of platelets per megakaryocyte will be increased. The tasks is summarised in a list below.

**Tasks**

- Fabricate systems according to design in PDMS
- Do computer simulations in COMSOL to see flows theoretically
- Create flows in the systems visualise the results
- Introduce Megakaryocytes into the system and visualize it

**Theory**

In this work a number of processes are encountered and they are explained in this section. Techniques regarding manufacturing and analysis are important to have some understanding of in order to be able to extract information and be able to change parameters correctly.

**Fabricating a master**

The master for the system designs is made by photolithography. Photolithography is a way to transfer a pattern onto a substrate by optical means. To be able to do this a suitable photoresist is needed. In this case SU-8 is used. SU-8 is an epoxy-based polymer which is sensitive to wavelengths in the range of 350-400 nm (UV-light).

To be able to attain a structure with high resolution a mask is needed, most often quarts with a chrome layer deposited as the wanted structure. Simplified, the procedure to produce a master consists of eight steps: Substrate treatment → Spin-coating of SU-8 → Soft bake → Exposure to UV light → Post expose bake → Develop SU-8 → Rinse and dry → Hard bake. With this done, a master is produced that can be used for further purposes[10].

SU-8 is a negative photoresist which means that when it is exposed to light in said range, the polymers will crosslink and is harder to remove. Then by putting the substrate in a specific chemical, the non-cross-linked SU-8 can be removed. Figure 4 illustrates how a negative photoresist can produce a structure through exposing it to light and then developing it [10].
Casting a system in PDMS

PDMS, short for polydimethylsiloxane is used for casting a system. PDMS is a polymer with a backbone of Silicon and oxygen. The process of casting a system in PDMS can be described in following steps: mixing elastomer and curing agent (10:1) → Moulding → degassing → curing → punch inlets and outlets → bonding. By mixing PDMS with a curing agent to make the chains to cross-link, a sturdy cast can be made with very small details transferred from the master to the PDMS structure. This is done by pouring the liquid mixture onto the enclosed master to stop the PDMS from floating away. After removing the trapped air bubbles the PDMS can be cured. Then after an oxidizing treatment of the PDMS surface, it can be bonded onto glass to complete the system [12]. An illustration of the manufacturing of the systems can be seen in Figure 5.

Figure 4. Illustration of a negative photoresist, by being exposed to certain light the polymers exposed will crosslink and withstand the developer and stay as the new structure. Source: [11]

Figure 5. A schematic illustration of the steps from manufacturing the master (step 1-2) to molding the systems in PDMS and bonding it (step 3-5). Source: [8]
Flow visualisation with fluorescence

Fluorescence is a phenomenon where light is emitted. As described in Figure 6. By adding fluorescein, a water soluble fluorescent powder to water one will get a fluorescent solution. This solution can then be used as one of the media when the systems will be tested. Then by using a special light source in the microscope the fluorescence can be seen. This will then show the flows in the system by different concentrations of light emitted depending on the flows. Another way to visualize flows is to use fluorescent particles.

![Diagram of excitation levels for a molecule](image1.png)

Figure 6. A model of the excitation levels for a molecule. High energy wavelengths is absorbed and low energy wavelengths (visible light) is emitted. Source: [13]

Vertical scanning interferometry

Vertical scanning interferometry is a way to measure differences in height and to get a visualized picture of a structure. This is done by scanning a structures step by step starting at the top. By adjusting the focus to different depths and measure the coherence of the light the scanning instrument can visualize the topography of the sample[14]. An example of this visualization is shown in Figure 7.

![Visualization of topography](image2.png)

Figure 7. An example of how the data from white light interferometry can be interpreted and the topography visualized
Experimental

Fabricating the master

The first master was used to mould the systems already designed. The mask needed to produce this master was already made and the structures can be seen in Figure 8 and Figure 9. Starting with a silicon wafer, a layer of photoresist (SU-8) is applied on top through spinning. Due to temporarily shortage of “SU-8 (50)”, an expired one was used for the first two tries. Then the wafer was heated on hot plates before being exposed in UV-light through the mask for defining the structure. The exposure was done with a mask aligner (“MA6 / BA6”, Karl Süss). After exposure, the structure was baked on hot plates. The structures were then developed with the developer “Mr Dev 600”. Ultimately the wafer was put in an oven for final treatment. More details of the fabrication can be found in recipe 1 and 2 later in this chapter.

![Figure 8](image)

Figure 8. An overview of the first systems produced. On top the general form of a system is shown with an outer and an inner channel, separated with 100 “chambers”. The differences in designs are the chambers and are shown in the bottom (1-8). In the bottom the left side represents the inner channel and the right the outer one.

The systems consists of two channels, one in the middle and one on the outside enclosing the inner one. The two channels are connected by one hundred chambers, fifty on each side of the inner channel. The inlets and outlets have a diameter of 1 mm.

Although the systems differ both in length and width, Figure 8 only shows an overview and the differences in the chambers. The designs have varying lengths of their structures and Table 1 summarise the dimensions. All eight designs are made so that the cells should enter the inner channel, get trapped in the chamber and protrude through the pore into the outer channel. Figure 9 describe in more detail where the channels are and how they are connected.
Table 1. The dimensions of the first eight systems are gathered in this table.

<table>
<thead>
<tr>
<th>System</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner channel (μm)</td>
<td>120</td>
<td>180</td>
<td>120</td>
<td>180</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Outer channel (μm)</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Pore width (μm)</td>
<td>4.5</td>
<td>4.5</td>
<td>2.5</td>
<td>2.5</td>
<td>4.5</td>
<td>4.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Pore length (μm)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Number of pores (μm)</td>
<td>1</td>
<td>6</td>
<td>10</td>
<td>12</td>
<td>1</td>
<td>6</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Distance between chambers (μm)</td>
<td>120</td>
<td>120</td>
<td>180</td>
<td>180</td>
<td>60</td>
<td>10</td>
<td>60</td>
<td>10</td>
</tr>
</tbody>
</table>

The outer channel will produce shear stress on the cells producing platelets and gather them. The two flows will have the same direction. Since the system is symmetrical it does not matter which direction it is.

Figure 9: Corresponding picture to the red box in figure 8. Showing how the chambers and pores (1-8) connect the inner channel to the outer ones.
The first master
The production of the first master followed the following standard recipe for SU-8. In Table 2 minor changes between tries can be seen:

Recipe 1 for spinning SU-8 resists

This recipe will give a film thickness of approx. 50 μm.

1. Dehydrate for 5 minutes at 200 °C on hot plate
2. Coating with SU-8 (50) through spinning.
   Ramp to 500 rpm at 100 rpm/s and hold 10 s
   Ramp to 2500 rpm at 200 rpm/s, hold 30 s
3. Soft bake on hot plate
   6 minutes at 65 °C then
   20 min at 95 °C
   wait for wafer to cool for 30 minutes.
4. Exposure in UV-light
   3*10 s with 30 s waiting time in between.
5. Post exposure bake on hot plate (PEB)
   1 minute at 65 °C
   5 minutes at 95 °C
6. Development
   6 minutes in SU-8 developer, Mr-Dev 600
   rinse in IPA
7. Hard bake in oven for 30 minutes at 150 °C

Two masters were made with help of this recipe. Changes from the recipe can be read in Table 2. Then the height was measured with a stylus profiler (“Dektak 150”, Bruker/Veeco). With an optical microscope and vertical scanning interferometry (“WYKO NT1100”, Veeco instruments) the masters were examined and the structures were visualised.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Try 1</th>
<th>Try 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of resist (ml)</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Max spinning speed (rpm)</td>
<td>2500</td>
<td>2750</td>
</tr>
<tr>
<td>Final film thickness (μm)</td>
<td>55</td>
<td>54</td>
</tr>
</tbody>
</table>

Process optimizing
Recipe 1 was made as a standard recipe. After manufacturing the master using recipe 1 and examined it, several batches of systems were made in PDMS. This resulted in poor quality of the structures, they were too high and not fully developed. The smaller details were not as designed and that is probably due to overexposure. A decision was made to optimize the process of making the master by changing the parameters in the process, such as spinning speed and exposure time.
This was done by first looking up information of the photoresist at the manufacturer[15]. Using their recommendations some changes in the process were made. Recipe 2 was made with the information gathered and masters were made. The new master (third in total) was examined in a microscope. Systems were then casted in PDMS using the master as mould. As new results was gathered minor changes was made to the process. Recipe 2 was used as starting point to achieve better results and with different tries some changes were made.

**Recipe 2 for spinning SU-8 resists**

This recipe will give a film thickness of 30 μm.

1. Dehydrate for 5 minutes at 200 °C on hot plate
2. Coating with SU-8 (50) through spinning.
   - Ramp to 500 rpm at 100 rpm/s and hold 10 s
   - Ramp to 4000 rpm at 200 rpm/s, hold 30 s
3. Soft bake on hot plate
   - 3 minutes at 65 °C then
   - 5 min at 95 °C
   - wait for wafer to cool for 30 minutes.
4. Exposure in UV-light
   - 2*8 s with 20 s waiting time in between.
5. Post exposure bake on hot plate (PEB)
   - 1 minute at 65 °C
   - 5 minutes at 95 °C
6. Development
   - 10 minutes in SU-8 developer, mr-Dev 600
   - rinse in IPA
7. Hard bake in oven for 30 minutes at 150 °C

Instructions from the manufacturer of SU-8 says that the use of an ultra sound bath in the developing step could give better developing[15]. This was done by having the beaker with developer and silicon wafer in it sunk into the water in the ultra sound bath.

Table 3. Showing the changes between the tries of making the first master with recipe 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Try 3</th>
<th>Try 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of resist (ml)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Exposure time (s)</td>
<td>2*8</td>
<td>12</td>
</tr>
<tr>
<td>Ultra sound bath</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Final film thickness (µm)</td>
<td>36</td>
<td>27</td>
</tr>
<tr>
<td>Evaluation</td>
<td>Useable</td>
<td>Not useable</td>
</tr>
</tbody>
</table>

Another two masters were made, but with recipe 2 as starting point. The changes in manufacturing between the third and fourth try can be read in Table 3.
The second master

New CAD designs were drawn, giving ten new designs (A-J). These were then printed into a mask with chrome upon quarts. The general form on the systems is still the same as the first set of designs. Only the chambers and pores are changed. In Figure 10 the new structures can be seen.

The new designs were made after analyzing the results from flow and cell tests that are presented during Results and Discussion. The reasons for creating new designs are described in the summary of Results and Discussion.

The new designs have fewer pores, one or two. The distance between chambers is either 20 μm or 10 μm. The pore width range from 1 to 3 μm and the pore length varies between 5 and 15 μm. Design D and E is also inverted and turn the inner channel into the one that collects the platelets. A total of three tries were made to produce these designs. In Table 4 the new dimensions for systems A to J are given.

![Illustrations showing the new designs of the chambers and the pores. Note that system D and E are inverted and is used when wanting the cells go from the inside to the outside.](image)

<table>
<thead>
<tr>
<th>System</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner channel (μm)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
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<tr>
<td>Outer channel (μm)</td>
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<td>20</td>
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<td>20</td>
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<tr>
<td>Pore width (μm)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Pore length (μm)</td>
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<td>10</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Number of pores (μm)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Distance between chambers (μm)</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In Table 5 changes between tries can be studied. Recipe 2 was used as base when producing the second master. There were some changes made to improve the quality of the master produced.
Table 5. Changes in parameter made from recipe 2 in the production of the second master.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Try 1</th>
<th>Try 2</th>
<th>Try 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft bake (65° C) (min)</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Soft bake (95 °C) (min)</td>
<td>5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cool down after soft bake (min)</td>
<td>0</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Exposure (s)</td>
<td>12</td>
<td>2*6</td>
<td>2*9</td>
</tr>
<tr>
<td>PEB (95 °C) (min)</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Development (min)</td>
<td>15</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Hard bake (min)</td>
<td>30</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Final film thickness (µm)</td>
<td>30</td>
<td>33</td>
<td>33</td>
</tr>
</tbody>
</table>

The process was as described for the first master. When developed, the height of the structures were measured with stylus profiler. The masters were then examined in a microscope and with Vertical scanning interferometry.

The changes in parameters for the new designs were made because the smaller structures were more sensitive to the internal tensions that exist in the SU-8. In try 1 the structures had cracked, so the changes were made to prevent the cracking as much as possible. In try 3 the cracks could still be seen but the master could still be used to produce systems in PDMS.

**Casting and Bonding of the Systems**

A series of systems was produced through casting, using the different masters as moulds. Polydimethylsiloxane (PDMS) was used as material. PDMS was mixed with a curing agent at the ratio 10:1. 30 grams of the elastomer PDMS was mixed with 3 g of curing agent. The PDMS was then poured over the master which was placed on a steel plate and had a steel ring around it to keep the PDMS in place. The master with PDMS was then placed in the fridge for approximately one hour to degas and remove all the bubbles before being placed in the oven at 75 °C for 60-90 minutes to cure.

After curing, the systems were removed from the oven. The four groups of systems were divided. Using a biopsy punch with a diameter of 0.75 mm holes for the inlets and outlets were punched in each of the systems. The groups of systems were then rinsed in an ethanol bath. The beaker with ethanol was placed in an ultra sound bath (5520, Branson) for approx. two minutes. The groups were dried with nitrogen gas before getting a corona (Heavy duty spark tester, Electro-teching products) treatment to oxidize the surface. The groups got the treatment for 10 seconds each. Microscope slides were also cleaned and treated with corona using the same parameters. Then the PDMS slabs were bonded to the glass simply by placing them onto the glass with the structures against the glass and gently press so there was contact everywhere. The bonded systems were then placed in the oven at 75 °C for 60 minutes for additional curing to harden the bonding.
Comsol simulations
The simulation program Comsol (Comsol inc.) was used to perform simulations of flows in the microfluidic systems. By importing the data files from Auto Cad the structures appeared in Comsol as 2D structures. Firstly simulations were done to see if the systems were drawn correctly. By varying the two flows a basic idea of how the flows behaved was formed. By combining this with simulations of particle trajectories indications of how the systems will work is achieved. Several simulations were done changing the ratio between the two flows in combination with closing and open the inner outlet. This was done to get information about the systems and what parameters could be used in the flow tests.

Parameters for a general simulation were set and the same simulation was done on all the systems to be able to theoretically compare them to each other. The flows were set to 0.02 μl/min in the inner channel and 0.005 μl/min in the outer. The height was set to 30 μm. Then the difference between having the inner outlet open versus closed was examined.

Visualisation of flow with fluorescence
An inverted microscope was used to evaluate the quality of the bonding between PDMS and glass. A fluorescent solution was prepared by mixing fluorescein and water, 0.1 g in 40 ml. Two 1 ml syringes where then prepared, one with water and one with fluorescein solution which is fluorescent. Then the syringe with water was attached to the central channel and the other to the outer channel. By using a needle of size G27 and a plastic tube with 0.1 inch inner diameter the syringe could be connected to the system. Various flow speeds combinations were tested as can be seen in Table 6. To achieve correct pump speeds syringe pumps of model “Phd 2000 Infusion” from Harvard apparatus were used. By doing series of tests where the flows are changing, the ratio between flows and its impact on the systems can examined. Also tests were done to investigate the flows when only the outer outlet was open versus both. The tests made were to find the most suitable flows to use when platelet production was tested. These tests were done only on certain systems, the ones that showed survived through the manufacturing processes. Not all systems had their structures fully developed and correctly bonded. The different flows showing in the tests are documented with a digital camera (Coolpix 4500, Nikon) mounted on the microscope.

Table 6. Different speed combinations tested using fluorescein in the outer channel. Inner outlet was closed.

<table>
<thead>
<tr>
<th>Inner channel (μl/min)</th>
<th>Outer channel (μl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

Fluorescent particles were used to do complementary studies. Particles with 3.2 μm in diameter were used in the first set of systems (1-8) and 1 μm in diameter for the second set (A-J). The particles were prepared by diluting 10 drops of particles to 4 ml with water, giving approximately 5*10^5 particles/ml. Tests were done with particles added to the inner channel to characterise and
show how the particles follow the flow and transfer through the pores. Pictures were taken and the information was noted. In Table 7 speed values for the particle tests can be found.

Table 7. Flow speeds used with fluorescent particles in the inner channel.

<table>
<thead>
<tr>
<th>Inner outlet status</th>
<th>Inner channel (μl/min)</th>
<th>Outer channel (μl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closed</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Closed</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Closed</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Open</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Open</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Open</td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

Platelet production test
The final tests for the systems were to see if Megakaryocytes can be inserted and if they produced platelets. This was done using cultured cells named Meg 01. The cultures are kept alive in incubators and when running tests the concentration is confirmed by the method of counting cells.

The first tests were done on system 1, see Table 1 for dimensions. Megakaryocytes were introduced to the system through the central channel as a floating suspension of cells in cell media. The concentration of cells was approximately 250 000 cells per millilitre. Cell media without anything extra was at the same time pumped through the outer channels. When loading the cells the flow speeds were 5μl/min in the central channel and 0μl/min in the outer one. The loading was done for 20 minutes. Then the flow speeds were changed to 5μl/min in the central channel and 3μl/min in the outer ones. During the studies of how the cells behave in the system the outlet for the inner channel was closed.

The systems from the new designs that were tested were system C, E-G and J. In Figure 10 and Table 4 the design details can be found. At first a cell concentration was approx. 500 000 cells per millilitre was used. The loading speed for the cells was 0.2 μl/min. Then the flow in the outer channel was turned on to 0.1 μl/min. The concentration of cells was then changed to 200 000 cells/ml and a new try was made. Different loading speeds were tested starting with 0.2 μl/min in the inner and 0 μl/min in the outer channel. By opening the inner outlet the cells can be “washed away” and the loading can begin anew. After loading of cells different speeds were tested. The different speeds tested can be seen in Table 8. The observations were noted and pictures were taken to save the results from the tests.

Table 8. Different flow speeds used in the study of the systems with cells.

<table>
<thead>
<tr>
<th>Part of test</th>
<th>Inner channel (μl/min)</th>
<th>Outer channel (μl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading of cells</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>Loading of cells</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Loading of cells</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Study of cells</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Study of cells</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Study of cells</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Results and discussion
Here the results will be presented and commented. The information achieved from the results will be addressed. The results will be presented in the different steps of the project for both old and new designs. Then a summary will follow. Do the systems work? What motivated the new designs? What could be improved? What future work is needed? These questions will be answered in the summary.

Fabricating the masters
When the first master was fabricated a standard recipe was used. After the fabrication the master was examined with a microscope, VSI and stylus profiler inspect to the resolution and height of the structures. The results can be seen in Figure 11 and Figure 12. The pictures show the chambers in each system.

As it can be seen in Figure 11 the smaller structures are not fully developed, this can be seen as the black areas in the images. There aren’t any structures in System 7 and 8. It is easier to see when compared to better structures in Figure 13. This poor resolution could be due to wrong conditions during the exposure. It could be over exposure. The light could have been diffracted and exposed larger areas than intended. These structures are high compared to the smaller details and could limit the size of structures that could be fully developed. Because of the poor resolution in the structures some process optimization were made. When looking at the pictures it can be seen that the small structures do not go through the resist down to the wafer. That makes making them of different depth than the larger structures. It is important to have the same height everywhere, otherwise there will be structures that do not bond with the glass in the bonding process. Figure 12 is made using VSI and shows the topography of the systems 1-4. The white areas indicate there are slopes that reflect the light away during the analysis. The red color represents the higher level and the blue the lower one. The results seen in the figure are a confirmation of the signs of poor quality of the structures given in Figure 11. In system 1 it can be seen that the space between the chamber and the outer channel is very thin and the structure is not fully developed according to planned design. The other small structures in the other systems are even less developed. In system 3 it is even hard to
notice where the structures forming the pores should be. It was believed that the process could be improved, but maybe it was expected since no specific recipe for these structures was made.

![Figure 12. VSI pictures of systems 1-4 from the first master. The red is representing the higher level and the blue the lower level.](image)

The recipe was therefore optimized. The parameters in the process were changed to improve the fabrication of the structures and achieve better resolution. Table 9 gives the changes made between the different recipes. With the new recipe the new designs were made. Pictures from the structures from the third try can be found in Figure 13. When compared to the earlier results the structures are of better quality overall. Results from the fourth try were not as good as in the third, where the big difference was the use of an ultrasonic bath in the fourth try. It is suspected that in this case with the ultrasonic bath damage the structures and lowers the resolution. The systems with one pore are clearly more detailed than before, the wafer can actually be seen between the chamber and the outer channel. The other structures is also of better resolution. In System 7 and System 8 the structures appeared. It can also be seen that the quality decreases with smaller structures. It can be seen primary in system 3 and 4 where only the outlines of the structures can be seen. In Figure 13 it can be seen that as the number of pores increases and structures becomes smaller the structures become less defined.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Recipe 1</th>
<th>Recipe 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second pinning speed (rpm)</td>
<td>2500</td>
<td>4000</td>
</tr>
<tr>
<td>Soft bake 65°C (min)</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Soft bake 95°C (min)</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Exposure (s)</td>
<td>3*10</td>
<td>2*8</td>
</tr>
<tr>
<td>Development (min)</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Expected film thickness (µm)</td>
<td>50</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 9. Changes between recipe 1 and recipe 2.

Probably it is as the structures becomes smaller the developer have a harder time of reaching down into the structures and develop, making the smaller structures undeveloped at the bottom. It is also possible that due to overexposure that the UV light reaches where it should not.
When looking at the aspect ratio of the structures in the third master, the height is 33 µm and the width of the smaller structures is 2 µm. This gives an aspect ratio of over 15. Structures of high aspect ratio are hard to fabricate. In the bonding process it was eventually discovered that System 2-8 never became good enough to make a system containing usable pores. To be usable the small structures need to be of the same height as the rest of the structures to bond correctly. This was the main reason to making the decision of reducing the number of pores to the next set of designs.

In Figure 14 VSI images of Systems 1-4 from the third master are shown. They also show that the structures are more developed and defined. Even if the structures are better, they still are not good enough. According to the results there are no vertical walls around the smaller pores, instead they...
are slightly inclined. There are no ways from this to tell how deep the holes are. In the bonding process it will be known if they are deep enough cause if they are, they will also bond to the glass.

Since the results from the fabrication got well enough for systems with few pores, the same process was used to produce the new set of systems. Results from the new designs can be found in Figure 15. Many dimensions of the new systems have been reduced to approx. one third compared to earlier and making the systems more condensed. This was done with a few different reasons that are described in the summary. The structures in these systems have been designed to have as big area as possible but still function as intended meaning that the cells should enter a chamber, protrude through a pore and produce platelets in the other channel. It was done with the idea that a bigger area and no thin structures would lead to better exposure and developing. As seen in Figure 15, the structures are very good and well defined. It can also be seen that all corners have been rounded. The rounded corners is probably due to overexposure in combination with not enough contact between resist and mask during the exposure. Only in one place was it found something not intended. In system I which has the smallest pore width is at 1 µm something different can be seen. The white spot in the pore is suspicious. By using the microscope and focus on the bottom instead of the top of the structures indications of the bottom structures are gone. Figure 16 illustrates the bottom of the structures in system C and I. System C is a reference and represents how the other nine system’s pores looked like. The pores in system I look like they are gone. Since they show when focusing on the top it is believed the pores have been developed so much at the bottom the SU-8 is removed and creating a “bridge” of SU-8 as a pore connecting the outer and inner channel.

![Figure 15. Images from an optical microscope of systems A-J. SA to the top left and SJ to the bottom right.](image-url)
Since the process did not get better than this it was noted that 1 µm was too small to be exposed and developed properly. One explanation to this is that the UV light did not reach through all of the resist leaving the bottom part exposed to developing. In this specific case a dose of 216 mJ/cm$^2$ was used. The manufacturer of the photoresist recommends a dose of 150-160 mJ/cm$^2$ this indicates of a slight overexposure and do not explain why the bottom have not been exposed. More probable is then that this high height compared to the structures lead to a gap between the mask and the lower part of the resist stopping the light from reaching all the way down due to diffraction. To be able to produce small structures the thickness of the resist also needs to be thin. The aspect ratio between height and size of the structures need to be reduced to produce smaller patterns. This is because the distance between the mask and the silicon wafer when exposing the photoresist. The longer the light travels through the resist it gets more diffracted and expose other areas than intended.

The fabrication of the masters started from scratch. It started with a standard recipe. The results from examination led to changes and process development. The results was achieved using both an optical microscope and VSI. The two methods give complementary data to each other and is used to confirm the results. Since the goal is to be able to run test with cells there are many steps to reach there. First the masters need to be made, then the casting of PDMS and even some flow tests to see if the system works and how they behave. When there are several steps using the results from previous steps it is very important to making the master as good as possible since the systems cannot be of better than the starting point.

**Casting and bonding of the systems**

The second step in producing functional systems is casting and bonding the systems. The masters is used as moulds and PDMS is poured over them and cure. The PDMS is removed from the master, cut into groups of systems and then bonded to microscope glass slides. Since the structures in the systems are so small, particles could easily sabotage the systems if it gets in the wrong place. Therefore it is very important to keep every surface as clean as possible to prevent this.

After the bonding process the result was examined in an inverted microscope. The images are inspected from below, through the glass slide onto which the PDMS is bonded. In Figure 17 images of the chambers from the first set of systems can be found. Suspected from earlier results is that the smaller structures would not bond to the glass. Looking at the chambers it seems that only system 1
and 5 is fully bonded. The smaller structures in the other systems seem to be out of focus or even gone which suggest that they did not bond to the glass.

![Figure 17. Images of bonded systems. System 1 to the top left and system 8 to the bottom right.](image)

In system 6 it can be seen that the walls separating the chambers have fallen. This is probably due to the thin structures and the walls are too heavy to stand stable on such small area. With these results only system 1 and 5 can be tested further with flows and later with cells. This strengthen the reason to why larger structures were designed in the second set of systems. Bigger structures are easier to produce and get good castings from.

The structures in the second set of designs were of better quality as can be seen in Figure 18 where chambers for systems C, E-G, and J are shown. The structures looked well defined with sharp edges except from the rounded corners. In system F where the pore length is the shortest (5 µm) the rounded corners affect the whole pore and no straight walls are constructed. It was noted that the pore width in system J is thinner than the others even though it is supposed to be 50% larger than the other ones. This is because the structures which are “pillars” made of PDMS are a little tilted. This caused the pore width to vary within each of the systems. These variations may not be a problem if designs with different pore widths should be compared with each other. Since the same variations were found in all systems then maybe it still is possible to compare them.
Is there any way to prevent the tilting of the PDMS structures? It is not certain if the problem occurs when removing the cast from the master or when bonding to the glass slides even though the suspicion lies with the bonding process. The structures and spaces in between are so small. It is very possible the structures tilt under the weight of the PDMS and the pressure applied during the bonding. It was tested to see if by bonding the PDMS differently would help counter this but it gave no change. If it is true that it is the pressure causing this then the use of some other technique could help and reduce the variations of the pore widths that are due to tilting of the structures.
Comsol simulations

By doing simulations in Comsol some descriptions of how the flows behave in the systems can be achieved. This was made for most systems but since only a few actually worked due to not containing any visible pores after bonding, not everything will be presented. This decision was made because there are no data to confirm or deny those simulations. The first simulations were done with the purpose to study the behaviour of the flow in the systems. It was detected that the flow tends to go through the inner channel shifting the flows toward the inner outlet. This was done on system 1 and can be seen in Figure 19. The three parts show the beginning, middle and end of the chambers. The squares in the overview shows were the images of the simulations are from. All simulations presented in three parts will follow this structure.

Figure 19. Flow simulation of S1 with both outlets open. From the left: Champers closest to the inlet, chambers in the middle, chambers closest to the outlet. The figure shows how the flows shift toward the middle channel and flow out the inner outlet. The boxes in the overview show where in the system the images were from.

These results show that the flows flow in the middle and not in the outer channels, where it was intended to flow to produce and collect the platelets correctly. The cells should be trapped in the chambers and protrude to the outer channels. For this to occur, the flows must shift toward the outer channels and not the inner. If it flows through the pores to the inner channel there will be tiny flows hindering the cells from entering the chambers. To counter this it was tested to do the same simulation but with the inner outlet closed to see what happened with the flow profile. The results were as expected that the flows now instead shift towards the outer channels. This can be seen in Figure 20. But the image describe that the flows in the first half shift to the central channel and in the second half shift back to the outer channels. If this is correct then the cells should not be able to
enter the chambers in the first half due to the small flow toward the centre. One should keep in mind that these simulations are done with completely open pores. In the cell tests the cells will clog the pores. This will of course change the flows towards other pores and affect the results.

This is good information to have in mind and can be of help to explain why the flows behave as it does in other experiments. These simulations were done with 0.1 μl/min in both outlets. With the inner outlet closed it was tested to double and threefold the flow in the central channel to see if a larger flow would create enough pressure to shift the flow toward the outer channels throughout the whole channel length. Even if the same event occurs with higher flow rate in the central channel one thing is noticed. The turn point, where the flow changes from going inwards to outwards is shifted closer and closer to the inlets. Probably a high enough flow rate would give a constant shift outwards.

![Flow direction](image)

**Figure 20.** Flow simulation of S1 with the inner outlet closed. From the left: inlet, middle, outlet. The figure show how the flows first shift toward the central channel then toward the outer channels.

To verify how this affects the movement of the cells, a simulation of how particles behave in the flow was done. By adding particles to the program it then calculates how the flow affects them and how they follow the stream through the channels. In this simulation the flowrate was set to 0.2 μl/min in the inner inlet and 0.1 μl/min in the outer one. As seen in Figure 21, the particles follow the flows quite well. By locking at how the particles flow, it is easy to see where the turning point (where the flows start to shift outwards) is.
Later the new set of systems was designed. The same simulations were done on them to see if the results were different. The new systems have smaller structures, thinner channels and smaller chambers. One of the reasons to shrink the width of the central channel so much was to raise the pressure in the central channel without needing to increase the flowrate. With these changes in the designs different result in the simulations are expected. The flows should now go towards the outer channels earlier in the systems than in the old designs. Figure 22 shows a flow simulation of system A with the flowrate set to 0.1 µl/min in both inlets and the inner outlet closed. The result of the simulation was that the flow is constantly shifted towards the outer channels confirming the expectations. According to Comsol, it does work to reduce the size of the central channel to change the pressure.
Figure 22. Flow simulation of SA with the inner outlet closed. From the left: inlet, middle, outlet. The figure show how the flows shift toward the outer channels.

Figure 23. A simulation of the particle trajectory in system A. From the left: inlet, middle, outlet.

With the constant shift of flow going outwards there is a much better distribution of particles over the chambers. Figure 23 shows a particle trajectory simulation in system A. As seen in the images, there are big differences compared to earlier systems. Now a very low percentage of the particles even reach the last chambers. They are instead spread over the whole interval of chambers in the system. To verify that the flow really is changing towards the outer channels through every chamber a simulation was made to visualize the pressures in the system as seen in Figure 24. The pressure difference between the inner and outer channels is approximately 7 Pa with the higher pressure in the inner channel. This support the earlier results saying that there is higher pressure in the inner channel.
Figure 24. Pressure simulation in system A. Illustrates the first chambers in the flow direction.
Visualisation of flow with fluorescence

Flow tests were done using a fluorescent solution in one inlet. By testing the first systems it was confirmed that the flow go toward the inner channel and out through the inner outlet. So it was tested to close the outlet on the inside and continue. As Comsol predicted, the flows still enter the central channel before being forced to the outer ones as seen in Figure 25. These images are from a flow test with fluorescein in the outer channels and the inner outlet closed. The flows are set to 5 µl/min in both inlets. The left picture shows the first chambers, then the middle ones and the left image shows the last ones. This could be corrected by increasing the flow in the central channel. In Table 10 a summary of how the ratios in flow rate between the inner and outer inlets affect the flow profile.

Figure 25. Flow test in system 1 with inner outlet closed. The left image shows the first chambers, the right shows the last chambers and the middle one shows chambers in the middle. In the overview it is shown were the images were taken.

The same test was done with the new systems and those results are also as COMSOL predicted. The dimensions have been reduced. In percentage the middle channel had been more reduced in width than the outer channels. This means that the pressure increase more in the inner channel than the outer ones. With this the pressure difference changes enough that it is higher in the central channel from the beginning as shown by Figure 24.

Flow tests were also done with fluorescent particles to see how they follow the flow. This is done to get some notice of how the cells will behave in the systems. Keeping both inlets open was compared to closing the inner one. The inlet speeds where in this test set to 0.2 µl/min in the inner channel and 0.1 µl/min in the outer channels. When both were open there was a huge loss of particles that just go straight through the central channel and out again. When the inner outlet was closed all the particles went to the outer channels, as wanted. This can be seen in Figure 26. The left image shows that particles fill the whole system and most are lost when they exit through the inner outlet. The right shows how the particles flow in the outer channels. There are particles in the inner channel from earlier and since there is now flow there they stand still.
Table 10. Shows flow ratios between the both inlets and their impact on the flow profile. A good flow profile entails low or no pressure difference between the channels making the flows stay in their designated channels.

<table>
<thead>
<tr>
<th>Flow profile</th>
<th>First designs</th>
<th>New designs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shifted to the middle</td>
<td>Inner flow &lt; 2*Outer flow</td>
<td>Inner flow &lt; Outer flow</td>
</tr>
<tr>
<td>Optimal</td>
<td>Inner flow = 2*Outer flow</td>
<td>Inner flow = Outer flow</td>
</tr>
<tr>
<td>Shifted to the outside</td>
<td>Inner flow &gt; 2*Outer flow</td>
<td>Inner flow &gt; Outer flow</td>
</tr>
</tbody>
</table>

Figure 26. Flow test with particles in system 1. The images show the channels after the chambers. To the left the inner outlet is open and the particles give rise to the blurry green in the picture. In the right the outlet is closed and the particles stopped in the middle were there is no flow.

The same results were found for the new systems and are shown in Figure 27. From this the conclusion that by keeping the inner outlet closed during the best result is achieved is drawn. The results from the flow tests tell us that the inner outlet should be closed to force the ells to enter the chambers. Otherwise most, if not all of the cells will pass right through the inner channel. The new designs should be better in cell tests because of the reduced sizes. With a constant higher pressure in the central channel the flow will go outwards without having to increase the flow rate. In a cell test that would mean that the cells spread out and enter chambers more evenly throughout the system. Wherein the first designs the cells would pass everything until the last chambers before entering a chamber. This gives a higher risk of clogging because the dispersion of flow to the outer channels is concentrated to a small length in the system.

Figure 27. Flow test with particles in system C. The images show the channels after the chambers. To the left the inner outlet is open and to the right it is closed.
Platelet production test

The platelet production test is the final test to see if the systems are functional and able to make the cells produce platelets. In the first set of systems only system 1 was tested. The other systems did not achieve the structures required to keep the cells in the chambers. It was quickly discovered that the chambers were too big dimensioned for the cells. The idea was that there would be only one cell per chamber. That is yet another reason why the designs were reduced in size in the new designs.

When system 1 was tested it was done with the inner outlet closed due to earlier results. The cells started to fill the system from the end to the beginning as suspected from the flow tests. This is shown in Figure 28. All the chambers from the end to the middle had at least one cell in them with the concentration of cells per chamber constantly decreasing.

![Flow direction](Image)

**Figure 28.** Cell test in system 1 showing that the cells fill the last chambers first then the last empty ones until everything is filled. From top left: Chambers closer to the inlet, chambers in the middle, the last chambers.

After the chambers got filled it was studied how the cells protrude through the pores. Many cells managed to squeeze through whole and follow the flow to the outlet. Eventually some cells was seen that extend an arm (pro platelet) out through the pore, exactly as they do when producing platelets *in vivo*. In Figure 29 it is captured when a cell had created a pro platelet through the pore. Unfortunately there was no evidence of any platelets being produced. The shear stress produced by the outer flow was not enough to rip of platelets from the arm. Instead it pulled the whole arm until the whole cell went through the pore.
The outer flow was probably too low to produce enough shear stress. But it was not possible to increase this without pushing the cells back into the central channel due to the needed flow ratio between channels. This could be solved by also increasing the flow in the central channel but it turned out it could not without casing other problems. The flow in the central channel needed to be almost at its minimum capacity to provide a flow slow enough to enable the cells to follow the flows and load the chambers correctly. When this limit the flow rate in both channels not enough shear stress is produced. By reducing the size of the outer channels which is done in the new designs a greater shear stress can be achieved by lower flow speeds.

When testing the new designs a very important discovery was made. There was debris of PDMS in the systems. After inspection it turned out that there has been debris in all systems. It is therefore suspected that the PDMS comes from the punching of inlets and outlets and the debris only became a problem when the channels had been reduced in size. In the first designs the channels was wide enough for everything to wash through when filling the systems. In the new designs the pieces of PDMS were big enough to clog the systems. Examples of this can be found in Figure 30. In some cases cells did enter the chambers and protrude as in the first design. Because of the problem with the PDMS no new results could be confirmed.
Summary
To tie everything together the results and major decisions will be presented. What motivated the new designs? Do the systems work? What could be improved? What future work is needed? These questions will now be answered.

There were a few reasons to why new designs were made. First, designs 2-8 had several pores that needed very small structures. These structures never got fabricated properly and did not give any useable system, see in Figure 17. Second, the cells were much smaller than anticipated and not dimensioned for these large chambers, see Figure 29. From these two reasons the design of the chambers were redrawn to become smaller and fewer pores so they can have as big structures as possible.

The flow profile in system 1 showed how the flow shifted towards the central channel before being forced out in the outer channels. This can be observed in Figure 25. By reducing the width of the inner channel the initial pressure will increase enough to counter the shift inwards and get the flow described by COMSOL in Figure 22. By doing this, it will become easier for the cells to enter a chamber because it do not need to turn as much in the flow to be trapped.

As mentioned in the cell test, the shear stress was not high enough to produce platelets. But since the flow cannot be increased due to the needed relations between inner and outer channels mentioned in Table 10 something else was needed. From this the width of the outer channels were reduced. That will increase the shear stress without increasing the flow. But wouldn’t that also increase the pressure and shift the flow inwards? Yes, it would but in this case the central channel is reduced even more and compensate for that and also changing the ratios needed between the two input flows.

As for systems D and E. Those are inverted in the designs intended to work in the same way but instead the cells would flow in the outer channels and enter the chambers from the outside. Then after protruding through the pore the pro platelet would be exposed to the sheer stress in the central channel. The idea was to see if it was possible to run working cell tests with both outlets open. If the flows behave as described in Figure 19 then the inverted systems have a good probability to work as well. But since the systems got clogged during the tests no results from this was achieved.

For the second question the answer is yes. The systems most probably work, with some changes made. The current results tell us everything works as intended except the actual platelet production.
This should be fixed by adjusting the flow outside the chambers which purpose is to rip of platelets from the Megakaryocytes. But since there was some problems with PDMS debris no working results was recorded.

What could be improved in future work? First and most important the PDMS debris needs to be removed. So in future work the major work should be to solve this first. If a new way of producing the inlets and outlets is found or some way of cleaning does not matter, as long as the debris is gone. If it is not solved then it does not matter what improvements are made since the systems cannot be tested correctly.

The next thing that could be done is to produce thinner structures and lower the height. By lowering the height the exposure in fabrication would be more precise because the distance between the silicon wafer and the mask is reduced. But more importantly, thinner structures would decrease the area of the pore and make it more probable that the cells stay in the chambers and protrude instead of squeezing through.

And of course, when these things are done the next step is to continue with the cell tests and find the parameters to get a functional platelet production started.

**Conclusions**

It is very important with the correct parameters when fabricating with UV-photolithography. The different baking times needs to be adjusted together with the exposure dose to suit the thickness of the wafer. Small variations could lead to big changes. The height of the fotoresist will limit the resolution of the structures. This is important to know so that all dimensions could be adapted to each other.

There will be PDMS debris in the systems when using a biopsy punch to create inlets and outlets. A way to create the inlets and outlets without debris needs to be found. At least a way to clean the systems before bonding them to glass needs to be found. Otherwise no further testing is possible.

More tests with cells are needed to find parameters that will make the platelets production work. The results gathered do not include a working production of platelets. The results are promising enough to say that they will almost definitely work with the correct parameters.


