Microfluidic Module labreport

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Abstract

In this module a microfluidic channel was fabricated so that it could be used to produce DNA-PEI polyplexes. Furthermore these were characterized by the technique called Nanosight. The results indicated that the entire procedure went as predicted and all parts worked smoothly. From the Nanosigh, a difference in the results of the diluted and undiluted samples was seen as the diluted samples gave higher concentrations at the entire size distribution compared to undiluted which gave only one major peak in concentration.

1 Introduction

The report contains both the aspects of the microfluidic module, meaning the microfluidic process as well as the nanosight part. B1 was the group behind the work and the microfluidic channel fabrication was followed by polyplex fabrication and characterization of polyplexes by the use of Nanosight. Furthermore it is the first part a total process that includes AFM characterization and particle analysis, with both of the later explained in later labreports.

2 Equipment

Silicon wafers, negative resist Su8-3050, MaskLess Aligner (MLA) 150, acetone, isopropanol, ethnanol, DI water, a spin coating machine, Developer mrDEV600, PDMS, gloves, hood fume, elastomer, curing agent, plastic spoons, desiccator, petri dish, oven and plasma oxidation machine.

3 Theory

3.1 Microfluidic channel fabrication

The motivation for using a microfluidic system is analogous to using integrated circuits to replace the discrete component circuits. The advantages are miniaturization, integration and automation. Some features with microfluidics are laminar flow rather than turbulent flow (meaning that the flow moves in one direction instead of several), high surface-to-volume ratio and very good flow control and particle manipulation possibilities.

The preferred method for implementing microfluidic properties is through a microfluidic channel. This is done to limit the fluid in question to the micrometer scale and to acquire mixing by diffusion. One gets a well-defined mixing time, highyl reproducible batches and fast production with the channel-method. Basically, two methods of mixing exists: passive, by diffusion in laminar flow and active, by external energy. Our focus will be on the first one.

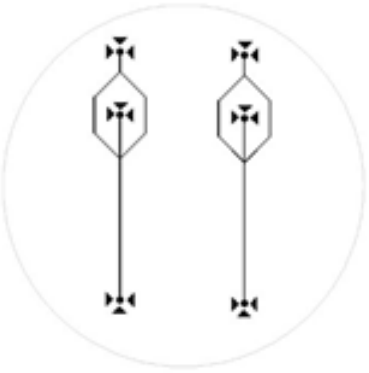

Figure 1: Schematic of the channels made. Input for PEI split into two channel and central input for DNA. The DNA and PEI are mixed in the central channel.

3.2 DNA-PEI polyplexes formation

A polyplex is any complex consisiting of a polymer and DNA. In this project the other polymer is polyethylenimine (PEI). The DNA is negatively charged and the PEI is positively charged. Both of the two components will self-assemble into polyplexes. An important aspect to consider in this regard is the ratio between DNA and PEI. Especially the amount of PEI is crucial; too much gives high toxicity, while too little is also undesired since one wants excess PEI.

Uses of this polyplex includes gene therapy , gene delivery systems. This is because it increases transfection delivery systems and still maintains biocompability.

3.3 Nanosight

Nanosight is based on the tracking of Brownian motion of nanoparticles in liquid suspension on a particle-by-particle basis. Brownian motion is the random motion of particles suspended in a fluid resulting from their collision with atoms. An alternative name to the phenomena, Nanoparticle tracking analysis (NTA) gives a good indication of how the phenomena is. Its working principle is based on light scattering and it is an alternative to the more traditional Dynamic Light Scattering (DLS).

The light scattering principle is based on light interacting with orbital electrons in molecules and induces dipoles. There are two types of scattering: Rayleigh scattering for nanoparticles with diameter much smaller than the wavelength of light and Mie scattering for nanoparticles with diameter roughly at the same size as the wavelength of light.

Nanosight furthermore simultaneously images and tracks each particle. It measures both size and concentration distributions. The tracking is done to determine the position at specific time intervals. Finally, some explanantion of terms is necessary: track = distance particle moves between frames and track length = total number of tracks 30 frames per second.

4 Methods

Methods part of the laboratory assignment consisted of 3 parts. These were: lithography, PDMS and 3D fabrication for creating a microfluidic channel device.. Two of them were done by group B1, while the lithography was done by the assistant. Below folliows an outline of the 3 processes. After this part, a DNA-PEI polyplex formation was done, before it was characterized by Nanosight. We will begin with the lithography:

In order to produce the mould, that will be used for channel fabrication later on in the process, a silicon wafer was covered with a negative resist referred to with its manufacturing name; Su8-3050. The resist was exposed without a mask, in stark contrast to the photolithography module where a mask was used. The actual process outline was as following:

1. Clean the Si wafer using acetone, isopropanol (IPA), ethanol and water
2. Place Si wafer in spin coater and add resist (25-30% of wafer should be covered)
3. Spin coat at 500rpm for 10s, then at 1900 for 40s
4. Bake at 65 for 30min
5. Expose wanted design using the MLA
6. Post exposure bake at 65 for 1min and 95 for 5min
7. Develop using mrDev600 for 15min and was with fresh developer IPA

The second part of the three-folded process was PDMS. As the reasoning for PDMS is already given in the theory-section, only the method will be elaborated on here.

1. Pour the elastomer in a plastic cup placed on the scale in the fume hood (record the weight)
2. Re-zero the scale
3. Pipette the curing agent/cross-linker into the same cup at 10% of the elastomer weight (in this case 25g : 2.5g should be enough)
4. Mix the two components thoroughly using the plastic spoons
5. Place the cup in a desiccator and leave for 20-30min
6. Pour the mixture onto the silicon mould in the petri-dish until the desired height is achieved
7. Remove as many excess bubbles as possible
8. Place in the oven at 65 for 3hrs
9. After curing take the PDMS out of the oven
10. Cut the wanted piece of PDMS (containing the microchannel) and peel it off the silicon (be careful not to break the silicon wafer)
11. Punch the 3 required holes (where they have been designed) for in/outflow of the liquid.

The final part of the assignment was 3D fabrication, where the intention is to bond it to an another material so that it could be usable. Due to this we have to bond them together with plasma oxidation as well. Both of these methods are outline in the next paragraph. 3D fabrication:

1. Take your PDMS pieces with the microfluidic channel and put it channel side up in the plasma ox.
2. Take your second piece and place it in the plasma ox.
3. Turn on (separate protocol)
4. Now put the two pieces together, making sure you do not touch the exposed sides and that the two sides touching are the ones that have been exposed to the oxygen plasma.

The final method is, as previously mentioned, plasma oxidation:

1. Switch on main switch
2. Insert sample
3. Turn on pump and ventilation
4. Press start
5. Hold until vacuum
6. Choose time using arrows and press reset for this to become the correct one
7. Choose gas you want (oxygen) and % (should be at 50%)
8. Set generator to 50% and push
9. Wait until vacuum is released
10. Remove samples
11. Relatively quickly place the two exposed sides together to produce an irreversible bond
12. Make sure all the buttons are in the out/off position
13. Switch off

The above was the method for creating a microfluidic channel. DNA-PEI polyplex procedure was also based on the same channel and the channel was used to create the polyplexes. The DNA and PEI were prepared according to a method and with values as according to the labtext. A complexation process were done in following order.

Complexation:
• Fill each syringe with 1ml of the solutions.
• On the pump set the volume to 600 uL and rate to 30 uL/min.
• DNA and PEI solutions should be flowing for approximately 3 minutes to obtain steady state before you start collecting samples.
• Use eppendorf tubes to collect samples – after 3 min switch to a new tube and collect the sample that will be used for analysis.

The final part of the experiment was something called Nanosight and was basically a characterization of our samples.

5 Results and Discussion

In this section a small walkthrough of the results will be done. Rather than focusing on the results, the emphasis will be put on answering the mandatory questions. The questions were formed into to 3 parts with each one of them classifying one part of the methods described in the previous section. These are: Microfluidic channel fabrication, formation of DNA-PEI polyplexes and size distribution of the polyplexes measured by Nanosight. We will therefore begin with the first part; **microfluidic channel fabrication:**

1. Write a short description of the fabrication

Group B1 developed a microfluidic channel device to be used to produce nanoparticles made out of DNA and PEI. The process consisted of 3 parts; lithography (done by the assistant), PDMS and 3D fabrication.

In the lithography part a maskless aligner was used. The intention was to produce the mould to be later used for channel fabrication. As usual in these kind of processes, a silicon-wafer was covered with negative resist and exposed to light. It should also be noted that since a negative resist was used, the exposed pattern will remain after the development. This gives a non-uniform size distribution on the wafer.

The second part was PDMS. Here two chemicals initiated a reaction to produce the final product, a polymer. This was done by an elastomer and a curing agent. The elastomer has the PDMS-polymer, while the curing agent is to solidfy the material.

Finally, the actual creation of the channel is commenced. This is followed by a 3D fabrication , where the channel is bonded to an another material like glass (the preferred material is our case). We used plasma oxidation to fulfill this task.

2. Discuss any problems that occured and how these were solved:

No significant problems occured during the process. Even the time aspect, since we had a compressive version of the module was negligble.

3. How does PDMS bond to the glass? The PDMS is bonded to the glass by the use of plasma oxidation of the two pieces and then comibining them. The plasma oxidation creates a covalent bond between the PDMS and the glass.

4. What would happen if you:

- changed the elastomer - curing ratio? If the of curing agent to elastomer is increased, a harder, more cross-linked elastomer results. If you decrease it would be too soft.
- curing temperature and time? Heating will accelerate the cross-linking reaction. The same goes for time.

The text will continue to the second part, **formation of DNA-PEI polyplexes:**

1. Write a short description of the procedure for the formation of DNA-PEI polyplexes

A polyplex of negatively chraged DNA and positively charged PEI will be created by the use of the microfluidic device we made in the previous section. When the two components get into contact with each other, they will instantly self-assemble into polyplexes (as indicated in figure 1). The polyplex-precursor were prepared according to the given values and ratio, before a complexation was done, as explained in the Method section.

2. Discuss advantages using microfluidics to produce such nanoparticles

Microfluidic in general gives a more precise control and manipulation of fluids that are at the sizedomain; nano-micro. It is also easier to obtain properties like automation and high-throughput. Although polyplexes can be made by other processes, the self-assembly is a bottom-up process and microfluidic with its dimension of the channel is ideal to control this. It gives the opportunity to analyze smaller volume samples and has a low reagent consumption. By the process we also get a

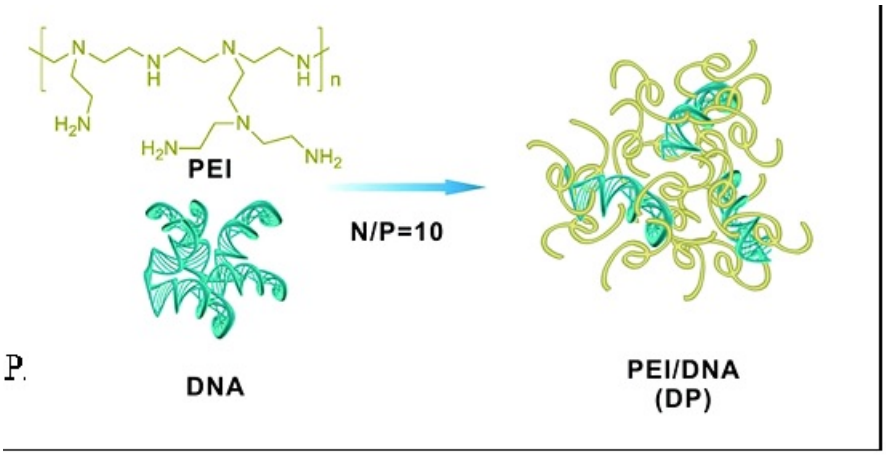

Figure 2: PEI structure and PEI/DNA complex

more homogeneous size distribution.

3. Discuss the impact of the parameter:

- flow rate: from Lu et al. it is suggested that higher flow rates gives enhanced mixing between the polyplex precursors. They attempted several flow rates and concluded that low flow rates were undesirable. A transfer of that result to this module should be done. However, too high flow rate could lead to leakage in the microfluidic channel, so a correct balance is required. The last aspect is that the mixing time is controlled by the flow rate.

- temperature: a slightly difficult question to answer, but the complexation happens at room temperature. The rest of the process is done in Nanolab, so temperature-wise the procedure for the lab are followed.

- solvent: the solvent change in quality leads to precursors forming nucleus during the change. These nucleus continue on to grow and self-assemble to nanoparticles. As done in this module.

- concentration of DNA and PEI, and the DNA-PEI ratio: the DNA-PEI ratio is important and interconnected with the concentration of DNA and PEI. This is because a high concentration of PEI could be toxic. Based on that, one tries to use as little PEI as possible, while at the same time using enough to have excess PEI. Basically a balancing act that has to be carried out. DNA and PEI concentrations should therefore be as indicated in the lab text. Not more, not less.

- length of the mixing channel and diffusion of DNA to achieve complexation: the length have to be of a certain dimension in order for the complexation to occur. If it is to short, then there won't be enough diffusion. This could be understood from the unit of the diffusion given in: cm^2/s.

The final part of the module was **measuring the size distribution of the polyplexes with the help of Nanosight**.

1. Briefly explain the principle behind Nanosight
The principle of Nanosight has already been explained in the theory section.

2. Present the size distribution results and concentration
The results will be presented below and the caption indicates the parameters like capture duration and dilution.

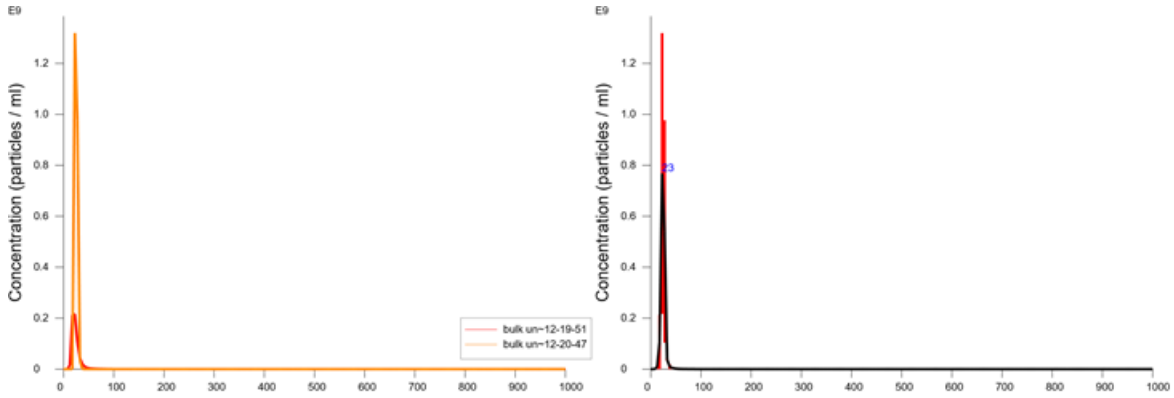

Figure 3: Bulk sample, undiluted with capture duration of 10 seconds

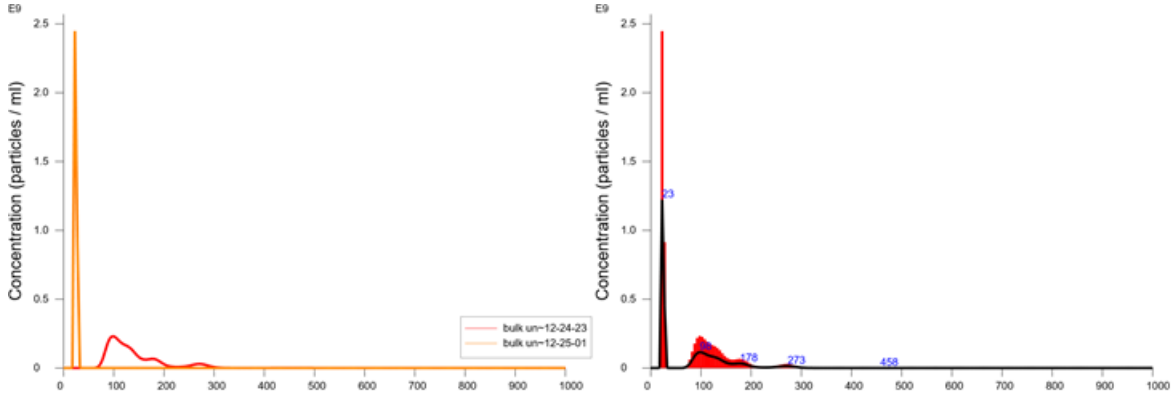

Figure 4: Bulk sample, undiluted with capture duration of 30 seconds

It should be pointed out that in this results (figure 5), there were some problems with transferring from the equipment's PDF-file and over to LaTeX. Since this was an anomaly compared to the other results, the result was included despite that.

3. Compare the size distribution of polyplexes of undiluted and diluted samples and discuss the result
The diluted sample 10 times increases the size of the polyplexes and gives a higher concentration at the latter ends of the size-distribution. While for the diluted 100 times this tendency is largely negated. It has one high peak (meaning concentration) at a smaller size and is kind of reminiscent

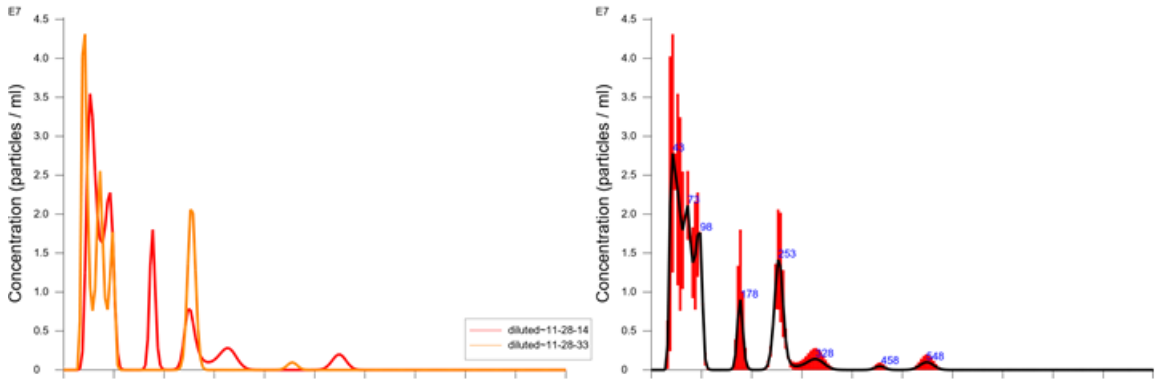

Figure 5: diluted 10 seconds

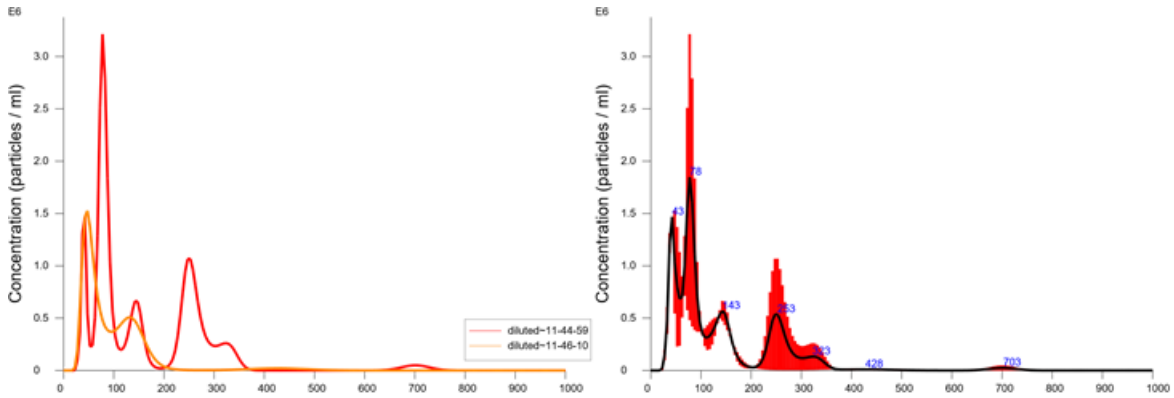

Figure 6: Diluted sample 10 times, with a capture duration for 60 seconds

of the bulk with a capture duration at 30 seconds. Although the concentration is higher. A final point is that there are higher peaks distributed more uniformly spread across the entire span.

The mean for the different sample is as following: diluted 10 times = 138.1 nm, diluted 100 times = 27.5 nm, bulk undiluted 10 s capture duration = 24.5 nm, bulk undiluted 30 s capture duration = 81.3 nm. The mean for the diluted 10 times is close to the bulk undiluted 30 s. One can assume that with a capture duration at 60 s, one would get closer to the value of 138 nm or something similar based on tendencies.

4. Comment on the results obtained using different capture duration (track length)
The capture duration clearly gives a larger span of the size distribution. This can particularly be seen on the bulk sample with 10 seconds and 30 seconds capture duration. In those mentioned samples capture duration is the only changing parameter.

5. Compare the size distribution formed by microfluidics and bulk mixing, and discuss the results

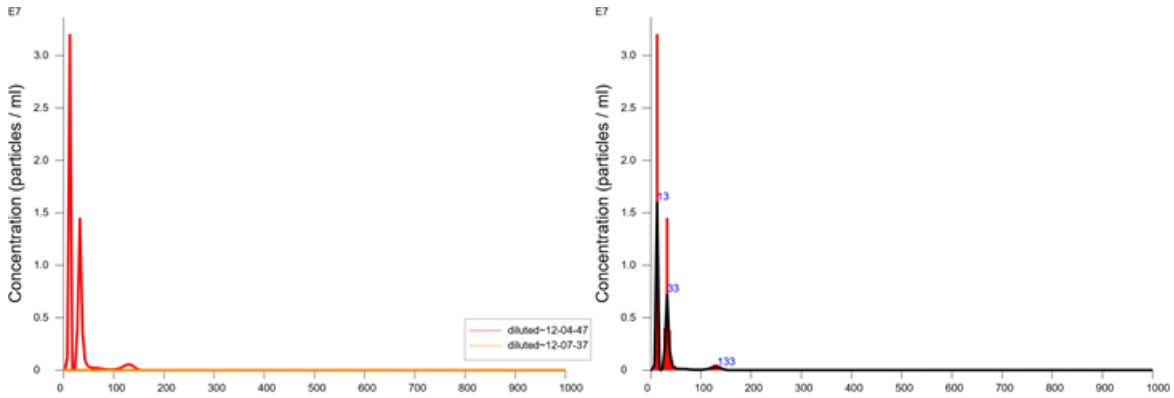

Figure 7: Diluted sample 100 times, with a capture duration for 60 seconds

We assume here that we are to evaluate the bulk samples that were created by microfluidics. This has partially been done above with regards to the capture duration. However, more discussion is necessary. The bulk mixing alone without dilution gives a peak concentration at 2.5 particles per ml at the smallest size. The other size's have low concentrations unlike the diluted ones. And there is one peak at the smaller size that is much higher than the other at the bigger capture duration. This is logical since no dilution gives that only one size is present. This can also be seen in the 100 times dilution (meaning excessive dilution) gives the same size distribution.

6. Discuss various reasons for not obtaining well-defined distributions
First of all it should be pointed out that the equipment has a standard deviation function that tries to limit the amount of errors in the algorithms of the equipment. This could be seen as a reason (if the SD is too high) and a way of eliminating the errors. Other reasons could be problems with the dilution of the sample, that could have gone wrong in some way. The dilution was done by us and a human error could occur. Several measurements of the same sample should also be done in order to remove errors or get results that are not abnormal. Due to severe time constraints for group B1, this could not be done. And backup tests were not conducted.

7. Compare the measured concentration of diluted and undiluted samples. Do the concentrations reflect the dilutions?
Also this question has partially been answered above. But, the measured concentration of the diluted sample 10 times gives a more uniform peak and higher concentrations at the different size's. The undiluted sample has one major peak at a small size. It has only smaller peaks at the capture duration of 30 seconds. Based on that, the concentrations reflect the dilutions.

6 Conclusion

In this report a microfluidic channel was developed in order to create polyplexes from DNA-PEI. This was so characterixed with Nanosight. All aspects of the process went as they should. The channel worked as it should and the polyplexes were formed. From the Nanosight part, a noticeable difference between diluted and undiluted samples was observed. The diluted samples gave a much higher and more uniform concentration of particles at various sizes. In comparison to the bulk undiluted sample that only gave one major peak at the lower end of the size distribution.