Love Wave Fully Integrated Lab-on-chip Platform for Food Pathogen Detection - LOVE-FOOD

(Contract No 317742 – Starting Date: 1 September 2012)

Deliverable 3.5

Nanotextured surfaces for DNA capture and related report

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29/09/2014	1.4	Final draft sent to FORTH
22/10/2014	1.5	Coordinator approves and submits to EC oficer

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Executive summary

In this deliverable we describe the design, fabrication, and successful demonstration of a sample preparation module comprising bacteria cell capture, and DNA extraction. Both submodules are based on capture of bacteria or DNA on plasma nanotextured surfaces. The module has achieved 100% efficiency for bacteria capture below a certain concentration (10^5 cells/ml), and DNA capture on surfaces containing COOH groups created by oxygen plasma. We have also proven that cell lysis can be performed on chip. However, this point will be discussed in a later progress report, to reduce the size of this deliverable.

Main text

1. Design and fabrication of bacteria capture and DNA extraction modules

1.1. Design version I

The NCSRD group designed and fabricated the first version of a cell capture and DNA extraction module. This chip design used classic concepts of on-chip chromatography and solid-phase extraction, and applies the novel plasma nanotexturing of polymers developed by NCSRD, a technology allowing increase of the surface area of the chip and the antibody binding capacity. The lithography mask of the two modules is shown in Fig. 1. The microchannels have embedded microposts in order to further increase the surface area and promote mixing.

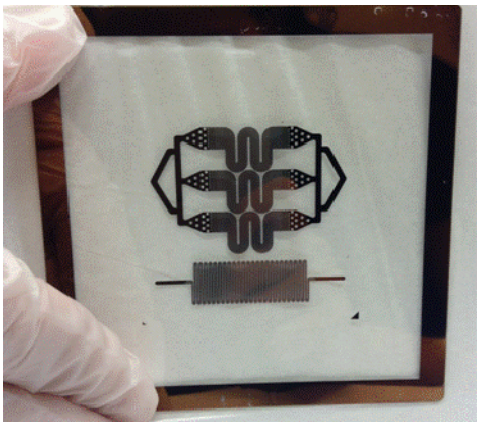
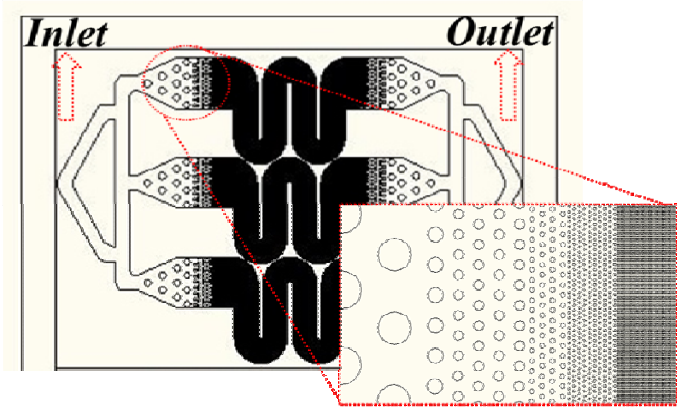


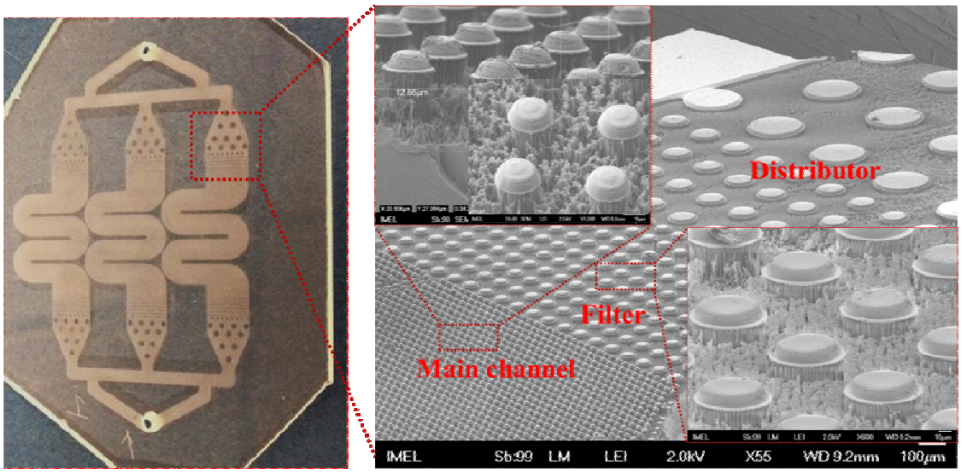
Figure 1. Lithography mask of the cell capture (top) and DNA extraction microfluidics (bottom of mask).

1.1.1 Cell capture module

The cell capture microfluidic is composed of 3 parallel microchannels with common input and output ports, which were fabricated by direct lithography and plasma etching on the PMMA substrate, and sealed with lamination film. The microchannels contain posts separated with distances larger than the bacteria dimensions. The intentionally created roughness by the oxygen plasma etching process resulted in a network of micro/nano textured, high-surface area microchannels, as the deterministic analogue of a porous monolith exhibiting a very high surface-to-volume ratio, and created the necessary chemistry for antibody binding. The chip is sealed with a lid, after bacteria capturing anti-LPS antibodies are immobilized on the chip bottom and walls. Heaters are included below the chip to allow in situ cell lysis. This first design has relatively large microchannels in order to allow larger flowrates and thus reduce the pumping time of the large sample volume (larger than 1ml). The bacteria capturing anti-LPS antibodies are immobilized on the chip bottom and walls. Fig. 2 (a, b) shows the layout of the chip design with 3 parallel microchannels, an optical image of the chip, and zoom in of the PMMA cell capture module after lithography and O_2 plasma etching.



(a)



(b)

Figure 2. (a) Layout of the chip design with 3 parallel microchannels. (b) Optical image of the chip, and zoom in of the PMMA cell capture module after lithography and O_2 plasma etching on 2mm PMMA substrates. The capture module consists of three parallel microchannels the contain posts in order to control the flow.

1.1.2 DNA extraction module

At first the DNA extraction module was composed of a coiled microchannel 500 mm long with common input and output ports, which were fabricated by direct lithography and plasma etching on the PMMA substrate, and sealed with lamination film. The extraction microchannels contained inside micro-pillar structures in order to increase the available surface area, thus enhancing the load of target material as well as enhancing the interaction probability between the solution borne nucleic acids and the nanotextured surface. The nanotextured surface inside the chip is shown in Fig. 3 together with an optical image of the DNA extraction module. However, the large length of the chip in this first design (500mm), the long flow through time (20min), the large number of turns, and posts, resulted in swelling of the lamination film with the PEG-ethanol solution. Thus, several chips had leaks after a few min of experiments. For this reason we were able to do only one experiment in each chip. Therefore, the DNA module was redesigned to avoid such leaks (see below).

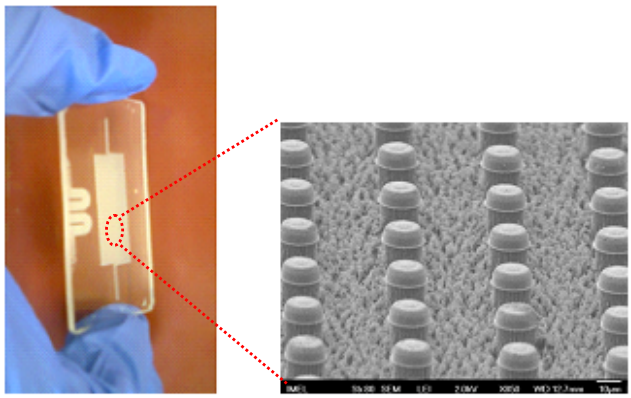


Figure 3. The nanostructured surface inside the chip.

At first a shorter microcolumn was used and different sealing methods were tested. The shorter plasma activated column was empty inside (without posts) and about 175um wide, 40mm long and 20um deep as shown in Figure 4. This column had absolutely no leak problems. However, this column was too short to allow binding of enough DNA to be detected by our optical absorption setup. In addition mixing of the DNA and ethanol solutions was not appropriate. Thus, the optimum DNA chip design is somewhere between the two designs presented above. This will be discussed below.

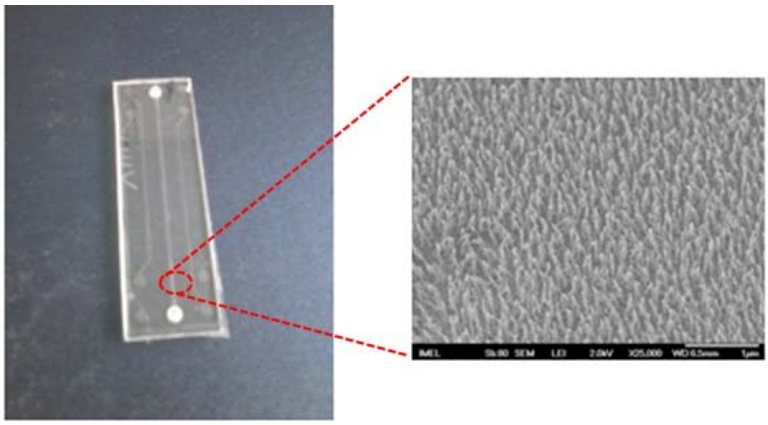


Figure 4. The short column and the nanostructured surface inside the chip.

1.2 Design Version II

1.2.1 Cell capture module

The first design (Design I) of bacteria capture module gave very good results. However, some geometrical modifications were found necessary for good sealing. The distance between the three parallel microchannels was increased in order to improve the sealing of the lamination film. Also posts were added in the inlet and the outlet of the chip in order to avoid blocking of the microchannels by collapse of the lamination roof. The chip length was too large for cells numbers less than 1000. However we decided NOT to reduce the length, as we anticipate that we could

increase the flow rate to reduce the flow through time. Alternatively we could use only one instead of three microchannels (see Fig 4b).

The optimized design with the new minor modifications for the cell capture module is shown in Fig. 5.

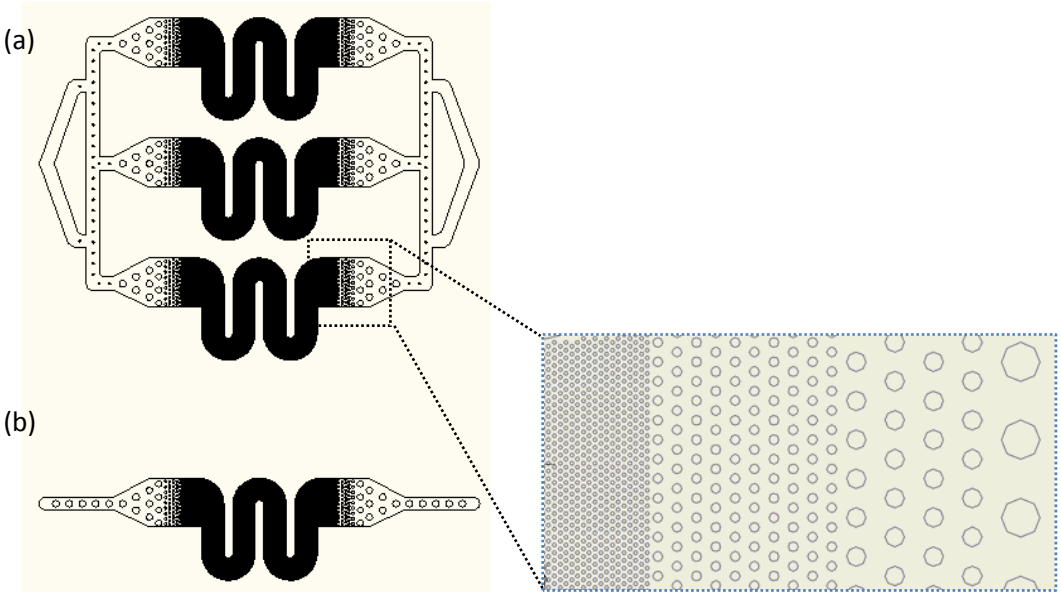


Figure 5. The new optimized design of the cell capture module.

1.2.2 DNA extraction module

The DNA extraction module was totally redesigned. The new design can improve the mixing of DNA with the PEG-ethanol solution and leads to a smaller channel that can withstand higher flow rates and thus can reduce the experimental time. The new design is shown in Fig. 6. The microchannel length is 400 mm and its width is 400 μm. No posts were included inside, since the new design is a mixer, and the flow is expected to be well mixed. The lack of posts will allow larger flow rates and smaller pressure drop. The short elbows, which are separated by enough distance is expected to eliminate any leakage problems from lamination failure.

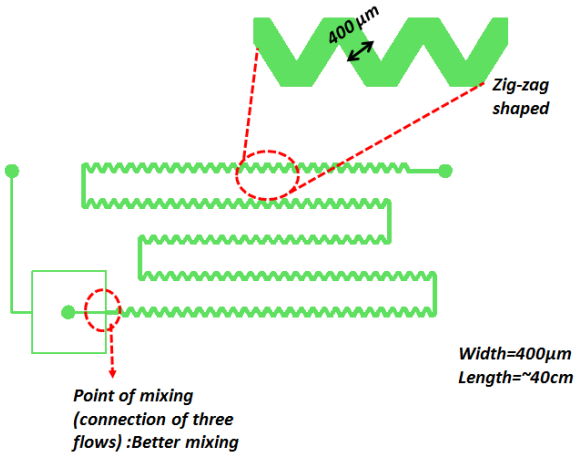


Figure 6. The new design of the DNA extraction module.

1.2. Immobilization of monoclonal and polyclonal anti-LPS antibodies on plasma nanotextured chip surfaces, and model assays to prove antibody efficiency

The surfaces of the fabricated cell capture chips were nanotextured in O_2 plasmas. Then the anti-LPS antibodies were immobilized on the plasma treated chips surface, without any linking groups. Immobilization takes place initially by adsorption and then by covalent binding using the active OH, C=O, and COOH groups on the plasma oxidized surface of the polymer. The strong immobilization has been proven by repeated and harsh washing steps, which do not remove the antibody from the surface. Additional experiments have proven that such immobilization is probably covalent. *NCSR Demokritos filed a patent for this new technology of antibody attachment on plasma nanotextured surfaces, entitled "Method to fabricate chemically-stable plasma-etched substrates for direct covalent biomolecule immobilization".*

The anti-LPS antibody binding process is shown below in Fig. 7, together with the demonstration of the antibody activity in a microarray format, and in a microfluidic format for nanotextured surfaces.

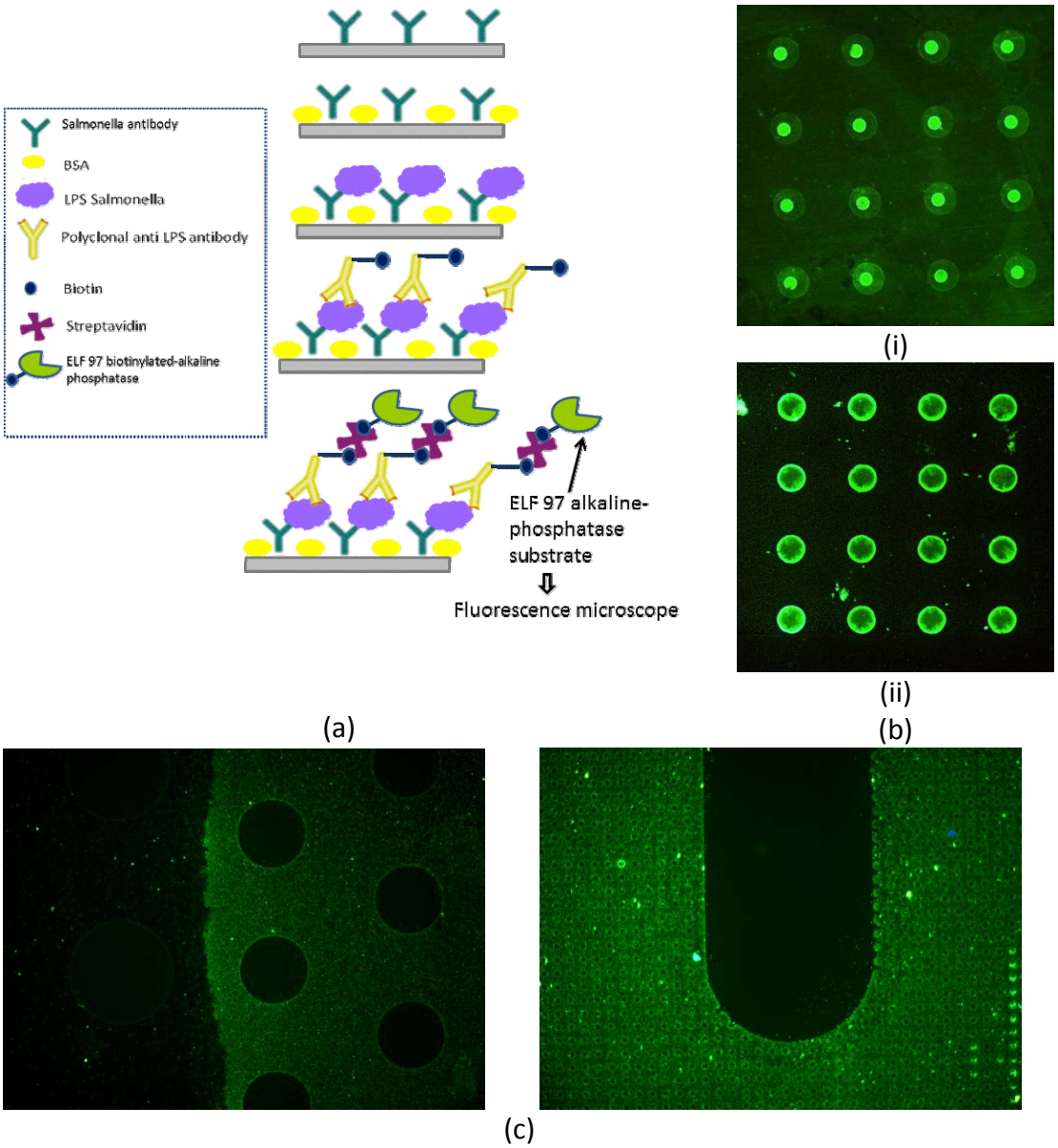


Figure 7. (a) Process of anti–LPS sandwich assay. (1) Coating with anti-LPS Antibody, (2) Incubation for 24h at RT, washing and immersion for 2h in a 10g/l

BSA solution, (3) 2 h Reaction with LPS solutions (0-500 ng/mL), (4) 2 h Reaction with biotinylated anti-LPS antibody and (5) reaction with streptavidin-alkaline phosphatase conjugate and finally, incubation with EFL 97 precipitate substrate. (b) Fluorescence images from microarrays on (i) untreated and (ii) O_2 plasma treated PMMA surfaces, coated with anti-LPS antibody after reaction with a 500 ng/mL LPS solution and detection as it is depicted in (a). (c) Fluorescence images from a microchannel coated with anti-LPS polyclonal antibody after reaction with a 500 ng/mL LPS solution and detection as it is depicted in (a).

2.1 Conclusions of testing phase

The two polyclonal antibodies from KPL and AbD Serotec Companies and the monoclonal antibody from MyBioSource Company were active and showed a strong signal after reaction with streptavidin-alkaline phosphatase conjugate and finally, incubation with EFL 97 precipitate substrate. Having proven that antibodies can be immobilized on the chip surface, and that they are active, the NCSR"D" group in collaboration with FORTH decided to perform experiments to prove that the Salmonella cells may be captured with efficiency using the cell capture chip module employing optimized protocols and three different types of antibodies.

3. Bacteria capture module testing with bacteria cells

3.1 Experimental set-up

The experimental set-up that used was very simple and consisted of a syringe pump in order to inject the salmonella solution inside the chips. A device holder was fabricated for holding in the bacteria module. PEEK capillary tubes (internal diameter 150 μm, external diameter 360 μm) and related connectors to the chip holder were used for inlet and outlet. Bacteria were introduced into the capture module at a flow rate of 1μL/min and the experiment lasted 100 min (injection of 100μl). Fluorescence images of the whole bacteria module were acquired with an epifluorescence microscope and at the end of the experiment the effluents were collected for conventional plate counting. Fig. 8 illustrates the microchip connected to syringe pump.

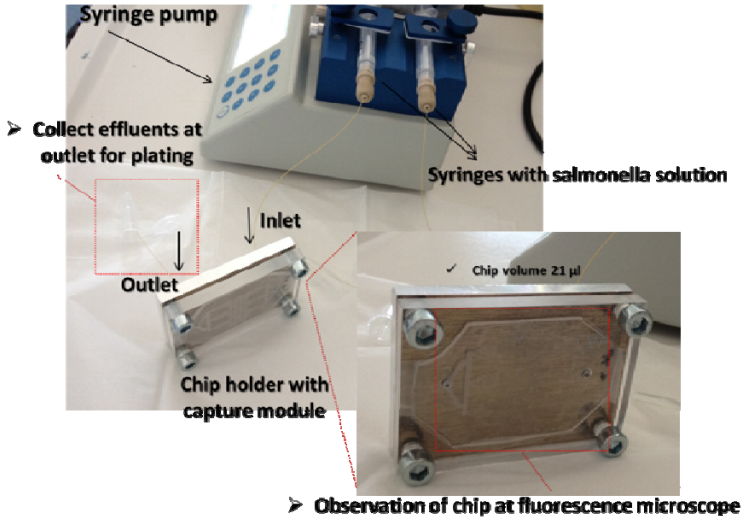


Figure 8. The microchip connected to syringe pump. The device holder with the capture module is shown in enlargement.

3.2 Direct detection of Green Fluorescent Protein (GFP)-labeled Salmonella cells on plasma nanotextured polymers

For the experiments we selected Salmonella Typhimurium (strain number 60.62) as aim bacteria strain and E. Coli as interfering bacteria strain. Both bacteria strains had been provided by Pasteur. The selected bacteria have been transfected to express GFP or RFP and thus to differentiate easily both bacteria strains in the fluorescence microscope for immediate observation and measurement. The bacteria were harvested by centrifugation (4.000 rpm for 4 min) and diluted in PBS to an initial concentration of approximately 10^8cells/mL from a culture in exponential growth phase in a LB (Luria – Bertoni) medium. The initial concentration was serially diluted with PBS to the desired concentrations. Optical density measurements were conducted at 600 nm for each one of the concentrations employed, and viable cell number was determined by conventional plate counting. The anti-LPS antibody binding process for direct capturing and detection of GFP – Salmonella or RFP – E. coli on PMMA chip bottom and walls is shown below in Fig. 9. We employed two polyclonal and one monoclonal antibodies (from KPL, AbD Serotec and MyBioSource Companies) targeting at the LPS core of the bacteria, prior to the addition of bacteria (Salmonella Typhimurium or E.coli). In order to confirm the attachment or not of the bacteria, SEM and fluorescence images after the completion of experiments were obtained.

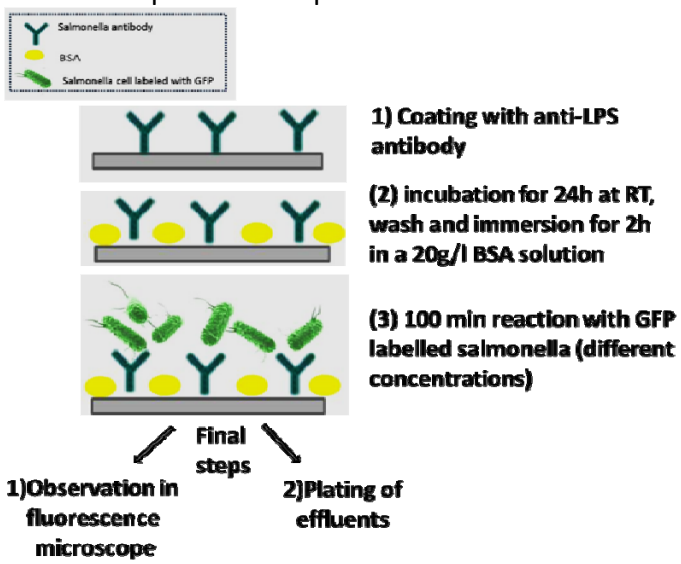
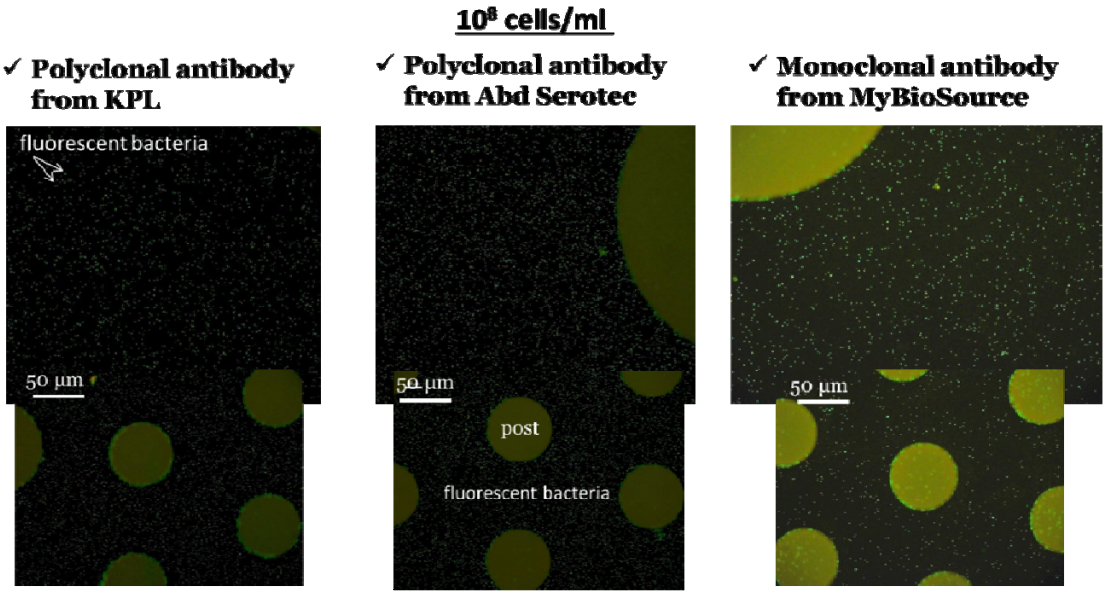
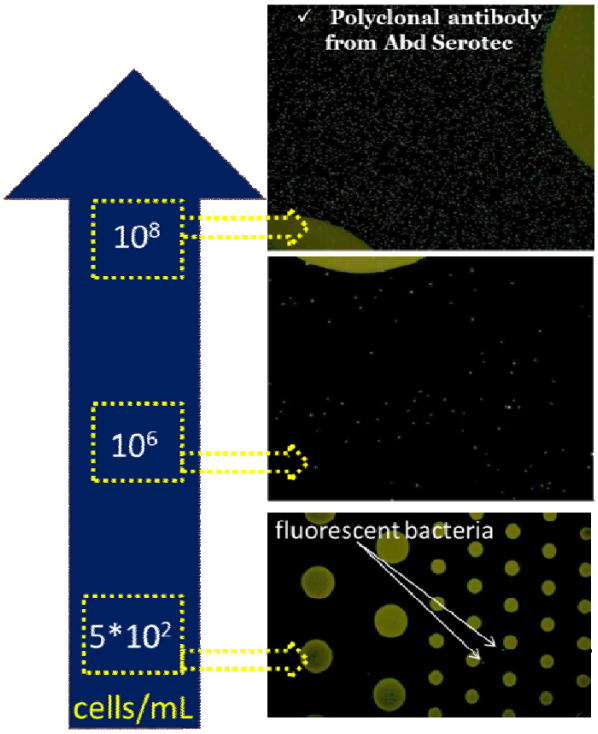


Figure 9. The anti-LPS antibody binding process on PMMA chip bottom and walls. 1) Coating with anti-LPS antibody, (2) incubation for 24h at RT, washing and immersion for 2h in a 20g/l BSA solution, (3) 2 h reaction with GFP labeled salmonella or RFP labeled e-coli cells (different concentrations), (4) washing and observation in fluorescence microscope.

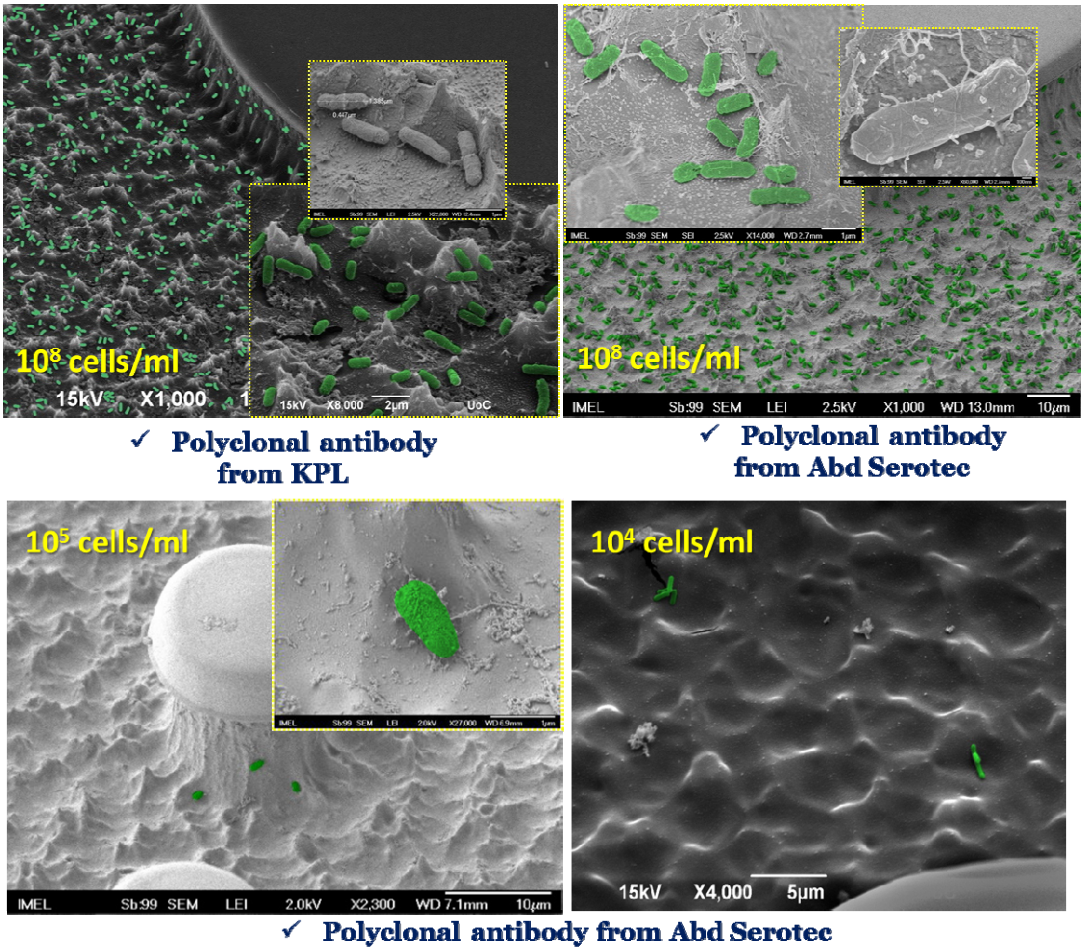
In Figure 9(a), representative fluorescence images of capturing and detection of GFP-Salmonella Typhimurium are shown after immobilization of different anti-LPS antibodies for concentration 10^8 cells/mL. Also in Figure 9(b) fluorescence images obtained from O_2-plasma-treated PMMA capture modules after immobilization of polyclonal antibody from AbD Serotec are shown for different concentration of bacteria varying from 10^2-10^8 cells/mL. By using the PMMA capture module, the Salmonella cells captured with high efficiency. In addition, in Figure 10(c) representative tilted SEM images of capturing and detection of GFP-Salmonella Typhimurium are shown using for example three bacteria concentrations 10^8, 10^5 and 10^4 cells/mL and for two polyclonal antibodies polyclonal antibody from AbD Serotec and KPL, respectively. To guide the eye, the bacteria were stained using the Photoshop software. The SEM images verified that bacteria attached to chip bottom and walls.


10⁸ cells/ml
✓ Polyclonal antibody from KPL
✓ Polyclonal antibody from Abd Serotec
✓ Monoclonal antibody from MyBioSource
fluorescent bacteria
50 µm
50 µm
post
fluorescent bacteria
50 µm

(a)


✓ Polyclonal antibody from Abd Serotec
10⁸
10⁶
5*10²
cells/mL
fluorescent bacteria

(b)



(c)

Figure 10. (a, b, c) Fluorescence and SEM images obtained from O_2-plasma-treated PMMA capture modules after immobilization of anti-LPS antibody from AbD Serotec, KPL and MyBioSource (100µg/mL) and for different bacteria concentrations ($5*10^2 – 10^8$ cells/mL).

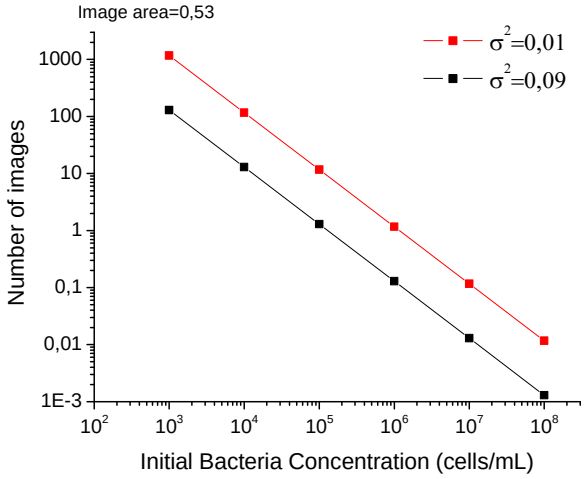
3.3 Protocol for fluorescence cell counting and efficiency determination

Because of the vastly varying concentrations used in these experiments, cell counting statistics are very different as one moved from high to low cell numbers. We needed to know how many photographs to take from a chip, to determine the capture cell number with a minimum accuracy (e.g. a minimum relative error). We thus developed a standard protocol of taking fluorescence images from the entire chip and doing the statistics of cell capture correctly. As you can see in the following equation, the number of images depends on the relative error, the area of the chip, the area of the image and the initial number of bacteria.

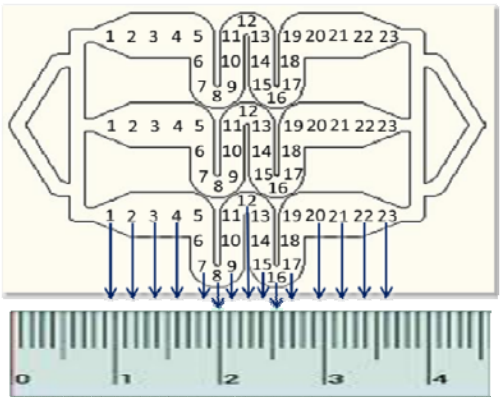
$$n_{images} = \frac{S_{chip}}{\mathbf{N}_{bacteria\ total} \cdot relative\ error \cdot S_{image}}$$

Where, n_{images} corresponds to number of images (photographs) that we should take in order to achieve the desired relative error, S_{chip} is the area of the empty chip, S_{image} is the area of the fluorescence image, and $\mathbf{N}_{bacteria\ total}$ is the total initial number of bacteria in chip. An assumption that we take into account is that the positions of bacteria on chip are uncorrelated, so we use the Poisson spatial statistics (complete spatial randomness). In Fig. 11 the number of

images is shown versus the initial bacteria concentration for two relative errors 10% and 90%. For relative error 10%, the number of images increases as the concentration decreases. For example, for concentration 10^3 cells/mL more than 100 photos should be collected to reduce the relative error down to 10%, something difficult by using conventional non-automated fluorescence microscopes without motorized XY scanning Stage. Therefore, the fluorescence microscopy is not recommended for small concentrations below 10^3 cells/ml. In our case after this study, we took 23 images in each microchannel, i.e. 69 photos for the whole 3-branched module taking into account that the relative error will be high for small concentrations, as shown in Fig. 11.



(a)



(b)

<u>Figure 11.</u> a) Required number of photographs for cell counting to reduce the relative error to values of 10% or 90% (two curves) versus cell concentration. b) Positions along the chip are shown with numbers. In these positions photographs were taken and analyzed, in order to have as accurate statistics as possible.

3.4 Results

<u>3.4.1 Direct counting of fluorescence images</u>

Counting of cell capture was done using Image J software. The image area was measured and converted according to the scale bar in μm^2 or mm^2. Therefore, cell capture density results as the number of the cells per square millimeter (cells/mm^2). By multiplying this with the area of the module the total cells in chip was estimated. The area of the module for the fluorescence images

is the area of empty chip. As seen from the data of Table 1 below, the estimation for small concentrations (<10^4) is poor. In Table 1 the results are presented for all three antibodies. The efficiencies are more than 90% for concentrations below 10^5 cells/mL.

Table 1. Counting of Data

Antibody	Initial Concentration (cells/mL)	Total Initial Number of Cells in chip	Total cells in chip	Capture efficiency (%)
Polyclonal: AbD Serotec	10^8	10^7	3610234	36
	10^7	10^6	508929	51
	10^6	10^5	75686	76
	10^5	10^4	9002	90
	10^4	10^3	1213	100
Polyclonal: KPL	10^8	10^7	2704840	27
	10^7	10^6	441924	44
	10^6	10^5	62480	62
	10^5	10^4	8858	88
	10^4	10^3	1730	100
	$5*10^2$	$5*10^1$	1294	100
Monoclonal: MyBioSource	10^8	10^7	853697	9
	10^7	10^6	188848	18
	10^6	10^5	39401	39
	10^5	10^4		
	10^4	10^3	1741	100

In Fig. 12 the capture efficiency calculated from fluorescence images is shown versus the initial bacteria concentration. The capture was quantified per area of the chip using the following equation.

$$capture\ efficiency = \frac{Total\ cells\ in\ chip}{Total\ initial\ number\ of\ cells\ in\ chip} \cdot 100\%$$

Based on the data the efficiency increases by decreasing the concentration and is approximately 100% for concentration 10^4 cells/mL and below. The capture efficiencies calculated from fluorescence images are slightly low, due to an underestimation that we think we have due to the sidewalls of the posts. The polyclonal antibody from AbD Serotec Company gave the best results.

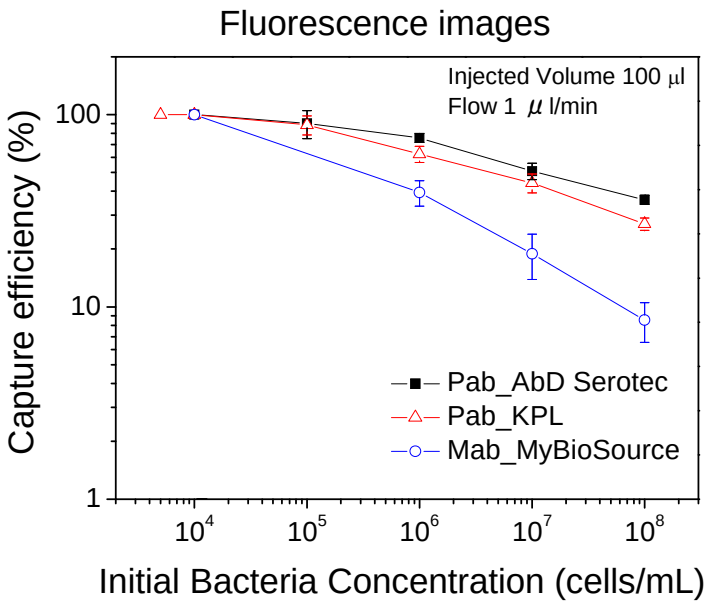


Figure 12. Capture efficiency calculated from fluorescence cell counting for three antibodies. We note that below 1000 cells/mL, we estimate more cells on chip compared to those injected. This is due to the poor statistics in this region. Although we collect 69 images, one would need 2-10 times more images for better statistics, see Fig. 10a. The errors correspond to standard errors based on three repetitions.

3.4.2 Plating of effluents

In order to determine the number of bacteria that are captured in the chip in a given population, we counted the bacteria by plating the effluents of the chip on media that supports the growth of the bacteria. Plating is the ideal method for enumerating microorganisms in a given population because it only identifies the living organisms in that population. The upper limit of accuracy for counting colonies is 300 colonies per plate (determined experimentally and statistically).

For the initial concentrations and the effluents we used serial dilutions, plating and counting of live bacteria in order to determine the number of bacteria in a given population. To this end we made serial dilutions, i.e. 1/10, 1/100, plated these bacteria and determined the total number of bacteria in the original solution by counting the number of colony forming units and comparing them to the dilution factor. Each colony forming unit represented a bacterium that was present in the diluted sample. The numbers of colony forming units (CFU's) were divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of bacteria per mL that were present in the original solution.

To calculate the number of bacteria per mL of diluted sample we used the following equation:

Colony count x dilution factor (the inverse of the dilutions) = CFU/ml of original culture

In Fig. 13 the capture efficiency calculated after plating of effluents is shown versus the initial bacteria concentration. The capture efficiency was quantified per unit volume of the microfluidic device. If we assume the inlet and outlet concentration of bacteria as C_{in} and C_{out} (in CFU mL^{-1}), respectively, the capture efficiency is derived as

$$capture\ efficiency = \frac{C_{in} - C_{out}}{C_{in}} \cdot 100\%$$

Based on the data the efficiency increases by decreasing the concentration and is approximately 100% for concentration 10^5 cells/mL and below, thus confirming the cell counting results shown above.

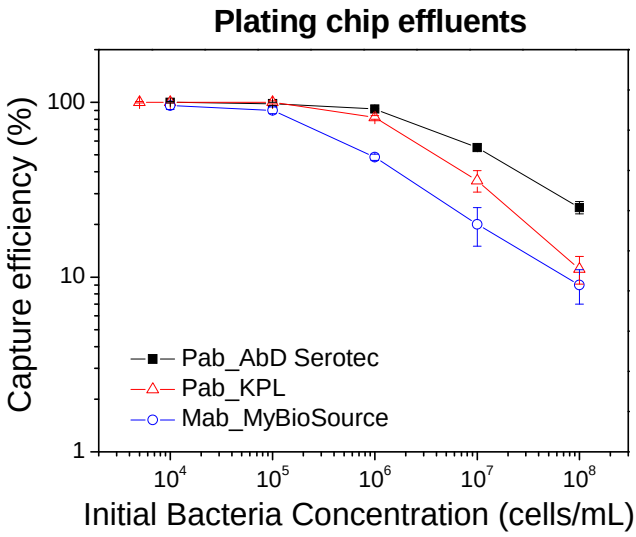


Figure 13. Capture efficiency calculated from plating of chip effluents for three antibodies. Note the agreement of plating results from those of on chip fluorescent cell counting shown in Fig. 12.

3.5 Specificity of Salmonella Typhimurium versus E-coli on PMMA cell capture microchip

Furthermore, we performed experiments in order to determine the specificity of Salmonella Typhimurium versus E. coli on PMMA cell capture microchips, pre-coated with 2 polyclonal and 1 monoclonal antibodies (from KPL, AbD Serotec and MyBioSource companies, one each time) (see Table 2). We used a mixture of bacteria 1:1 with concentration 10^8 cells/mL (GFP-Salmonella green and RFP-E. Coli red). As shown in Figure 14, using the cell capture module; we have been able to capture specifically Salmonella in a sample containing interfering bacteria with excellent selectivity (over 300:1 in all antibodies used) of salmonella versus e-coli for a 1:1 mixture (10^8 cells/mL). The results are very promising and ongoing experiments are evaluating the module in terms of capture specificity of salmonella versus lactobacillus bacteria often used in probiotic products.

Table 2. Specificity of Salmonella Typhimurium versus E. coli on PMMA cell capture microchips

Cell [mixture (1:1)]	Initial Concentration (cells/mL)	Injected Volume (µl)	Total Initial Number of Cells in chip	Initial cells/mm^2	Cells/mm^2	Selectivity
Monoclonal antibody MyBioSource						
Salmonella (green)	1,00E+08	20	2,00E+06	3,16E+03	2,77E+02	492:1
E-coli (red)	1,00E+08	20	2,00E+06	3,16E+03	5,63E-01	
Polyclonal antibody AbD Serotec						
Salmonella (green)	1,00E+08	20	2,00E+06	3,23E+03	3,92E+02	500:1
E-coli (red)	1,00E+08	20	2,00E+06	3,23E+03	7,88E-01	
Polyclonal antibody KPL						
Salmonella (green)	1,00E+08	20	2,00E+06	3,23E+03	5,38E+02	330:1
E-coli (red)	1,00E+08	20	2,00E+06	3,23E+03	1,63E+00	

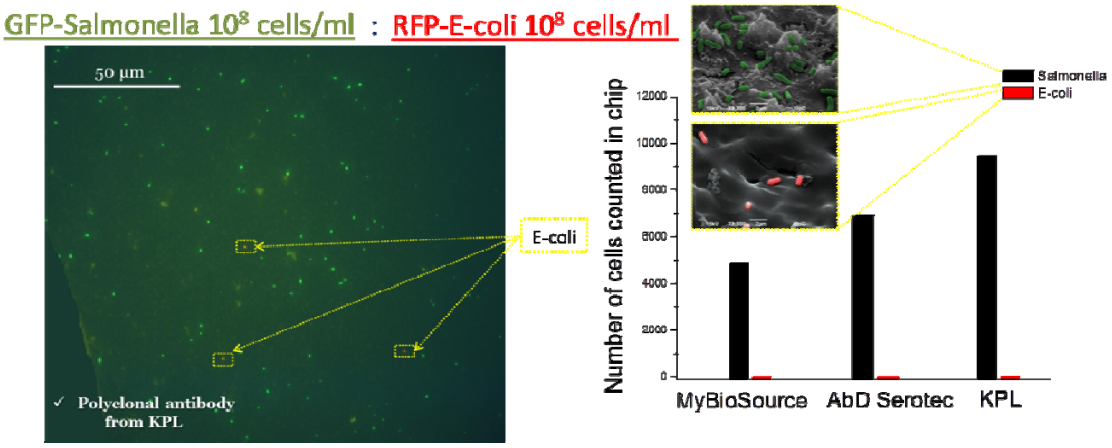


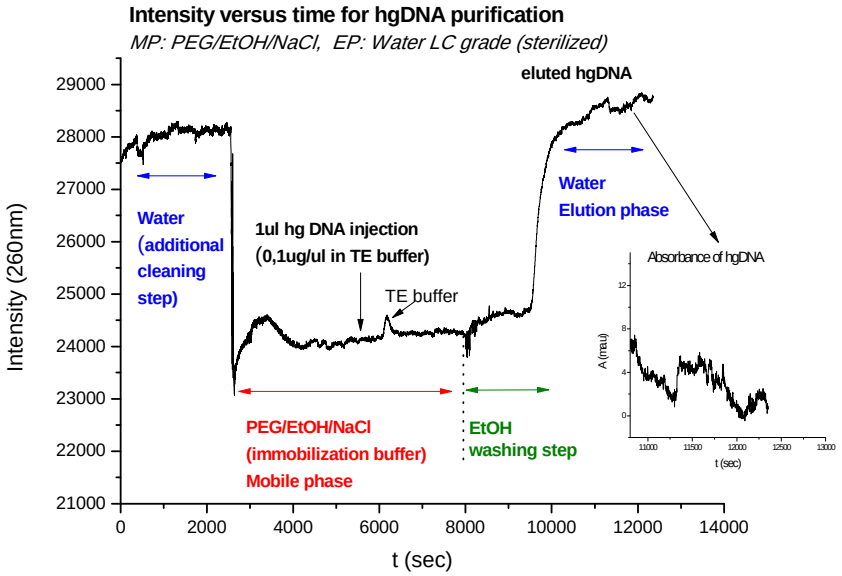
Figure 14. Specificity of (a) Salmonella Typhimurium versus (b) E. coli on PMMA cell capture microchip. Mixture of bacteria 1:1 with concentration 10^8 cells/mL (GFP-Salmonella green and RFP-E. Coli red).

4. DNA extraction module

4.1 Preliminary DNA extraction experiments using the first design

The surfaces of the fabricated PMMA chips for DNA purification were nanotextured in Oxygen plasmas and thus active OH, C=O, and COOH groups were created. Buffers were optimized for the process below after literature search. Then a solution containing 3% PEG (M_w =8000), 0.4M NaCl

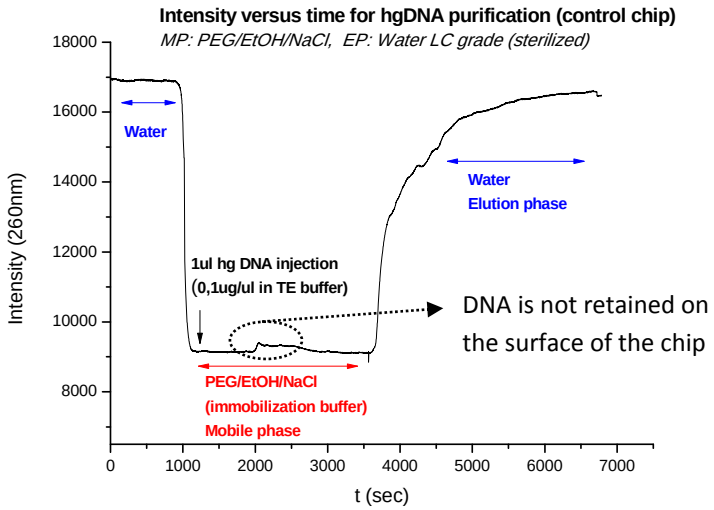
and 70% Ethanol was run through the chips and used as immobilization buffer. Thereafter hgDNA (0.1µg/µl) plug injection was done and after binding on the surface, an intermediate washing step with 85% Ethanol was performed. In the end, hgDNA was eluted with water of LC grade (first sterilized). The protocol is shown within the UV absorbance spectrum in Figure 15 below, which represents a real experiment versus time.



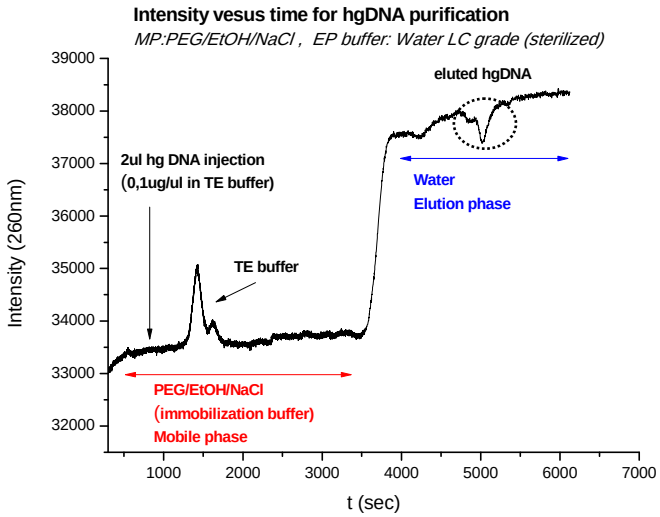
<u>Figure 15.</u> Intensity of detector for preliminary test of hgDNA purification on a nanostructured chip (7days aged chip).

<u>4.2 Comparison of plasma activated and non-activated chips</u>

After preliminary DNA purification experiments, the DNA purification module appeared to function and the hgDNA binds to COOH functional groups with the aid of precipitants PEG and Ethanol. Human genomic DNA purification was also examined on non-nanostructured PMMA chips *(control chips)* in order to verify that no DNA absorption occurs and the results were examined using UV absorbance at 260 and 280nm. These first control experiments were successful and absorption was not observed as shown in Figure 16 (a, b).



a)



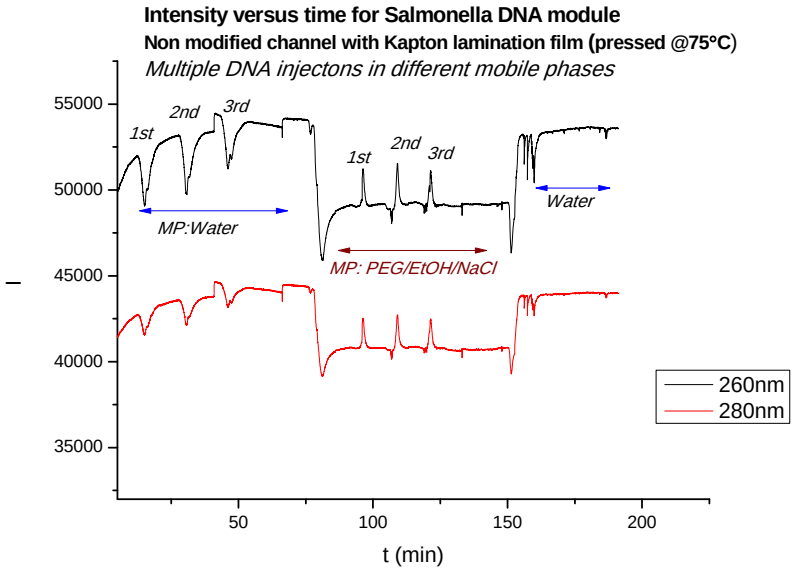
b)

<u>Figure 16.</u> Intensity of detector for preliminary test of hgDNA purification on (a) a control chip (NON plasma textured) as compared to (b) a plasma nanotextured chip. The proof of concept that DNA is bound on Oxygen plasma nanotextured surfaces is thus confirmed by the experiments shown in Fig. 15 and 16.

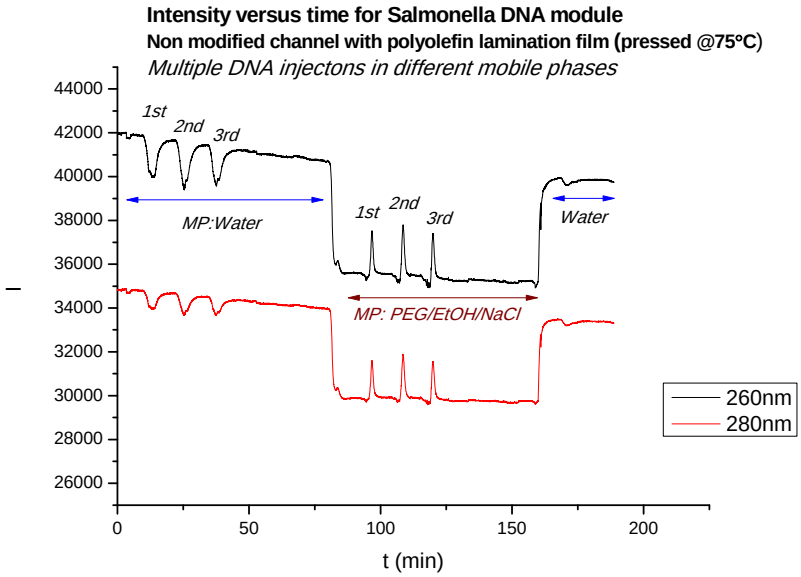
<u>4.3 First experiments in a simple layout to optimize sealing methods</u>

Having proven that COOH containing oxygen plasma nanotextured surfaces bound hgDNA, we went of Salmonella DNA. First Salmonella DNA purification experiments were performed with prepurified Salmonella DNA on nanostructured PMMA chips and the results were examined using UV absorbance at 260 and 280nm. Problems occurred with the reproducibility of the experiments because of swelling of the lamination film that was used for sealing the chips. The swelling observed (which happens many times but not always) is believed to be the result of ethanol use

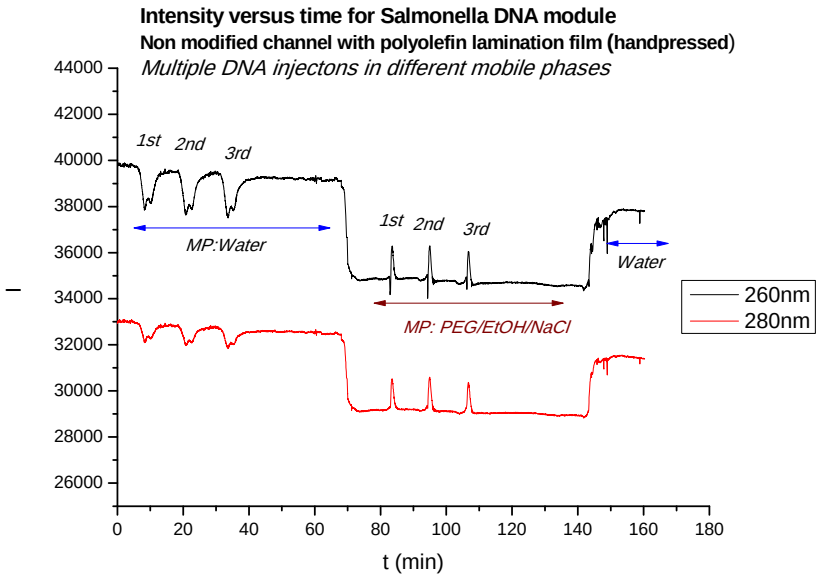
within the protocol of purification coupled with the vulnerable adhesive of the lamination film *(though not stated in its specifications)*. We decided to use thermal bonding as an alternative for sealing and experiments towards that direction were performed using a Carver Heat press. However due to technical problems with thermal bonding, we turned to lamination films.
Therefore we abandoned for a while the long DNA enrichment chip design and used short straight channels (see fig. 4 above), on which the lamination bonding strength was tested. For that purpose we used three chips that were not modified with oxygen plasma. The first chip was sealed with a Kapton lamination film via a laminator at 75°C, the second chip with a polyolefin lamination film via a laminator at 75°C and the third one with a polyolefin lamination film via hand pressure. Afterwards we ran three successive Salmonella DNA injections through each of these three short columns at first with water LC (first sterilized) as the mobile phase and then with 3%PEG/ 0,4M NaCl/ 70%EtOH as the mobile phase. The results are shown if Figure 17.



a)



b)



c)

<u>Figure 17.</u> a) First chip- Kapton lamination film@75°C, b) Second chip - Polyolefin lamination film@75°C, c) Third chip- hand pressed polyolefin lamination film. Repeated injections are shown simulating multiple real experiments.

The best sealing method was for the second chip that was sealed with the polyolefin lamination film via a laminator at 75° C. The PEG/EtOH phase was passed through for at least 1h for 3 DNA injections, while only one injection could be done with the old column (during an hour). Having selected the best procedure to laminate the chip, we went ahead to redesign the DNA module so that a better mixing of the immobilization solution and the DNA sample can take place, while the chip can withstand higher flows. The new design is shown in Fig. 6. As this report is being written the mask has arrived at NCSRD and results with the new mask will be reported at the next report.

Conclusions

Microfluidic chip for cell capture was designed and fabricated on PMMA. Plasma nanotexturing was used to increase surface area for improved antibody immobilization. Monoclonal (Mybiosource), polyclonal (Abd serotec), polyclonal (KPL) anti-LPS core Salmonella antibodies were tested successfully. The plasma nanotextured cell capture module was tested with various bacteria concentrations ranging from 10^2-10^8 cells/mL for monoclonal and polyclonal antibodies from Mybiosource, KPL and AbD Serotec Companies. We developed a standard protocol of taking fluorescence images from the entire chip and performed the statistics correctly. We also performed plating of the effluents of the chip for a complete characterization of the capture efficiency. Capture efficiency and selectivity of Salmonella versus e-coli have also been tested in all three antibodies. Sufficient capture efficiency (100% for concentrations < 10^5 cells/mL) was observed. In addition, excellent selectivity (over 300:1 in all antibodies used) of salmonella versus e-coli for a 1:1 mixture (10^8 cells/mL) was perceived. Finally, we submitted the Greek patent entitled "Method to fabricate chemically-stable plasma-etched substrates for direct covalent biomolecule immobilization" for the technology of antibody attachment on plasma nanotextured surfaces.

The DNA enrichment module was fabricated and tested successfully for hgDNA, and first experiments with bacteria DNA were done. However, leaks of the lamination film did not allow repeated injections in one chip. Efforts with much shorter chips showed that lamination with heating improve the sealing tightness; while a good mixing is needed during injection. To address these issues a new design of the DNA chip enrichment was done, and is presently being fabricated and tested.

On the whole, the approach of using nanotextured surfaces has proven extremely successful for the project and will be pursued further targeting integration with the other modules.

Finally, it should be mentioned that on-chip lysis was successfully performed, and was followed by successful off-chip DNA amplification. However, to reduce the already large size of this deliverable these results will be presented in a future report (D3.7). In addition, work on alternative strategies is still under progress; therefore, evaluation and decision on the strategy chosen will occur on M30, thus postponing MS13 (due month 24) to month 30.