Automated Modular Bacterial Filtering System With Embeddable Microfluidic Chips

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***Abstract*— This paper introduces an automated bacterial filtering system which has three unique advantages comparing to current available systems. Firstly, it is an automatic system which minimizes the human interaction with potentially hazardous bacterial samples, eliminates human errors and makes it suitable for frequent bacterial filtering procedures. Secondly, it provides an interface between milliliter volumes of sample and microfluidic chips requiring samples in microliter volumes. Thirdly, both bacteria collected on the filter and filtrate passed through the filter can be collected for analysis. The modular design of the system provides a large variety of filters for different applications.**

I. INTRODUCTION

Filtering of biological species such as bacteria [1][2][3][4] and blood cells [5][6][7] is a frequent and inevitable part of medical and biological laboratory procedures. A large number of methods and microfluidic approaches have been developed during the years. A microfluidic dielectrophoresis filter is used for separation of biological species [1]. Using of mechanical filtering weirs in a filter-based microfluidic device as a platform for immunofluorescent assay of microbial cells has been previously reported [2]. An in-channel 3D micromesh structure [3] and an integrated vertical screen microfilter system using inclined SU-8 structures [4] can be used for μTAS applications. Embedding silica microsphere filter in a chip is a way of blood serum separation [5], which facilitates the design of a disposable microfluidic filtering chips. Weir-type filters with interdigital aligned full-polymer microfluidic channels is another method of blood cell fractionation [6], which is exploited to filter whole blood cells from blood sample. Blood/Plasma separation based on bent microchannel structures coalesces two separation mechanisms, centrifugal force and plasma-skimming effect [7]. Many of these filtering methods are complex, costly, have very low throughput and require complicated fabrication procedures and their use is limited to a specific range of sizes of biological species. Considering prevalent methods of bacterial filtering systems, none of them is suitable for frequent filtering applications and they are designed for micro-scale applications. Bacterial filtering applications require high throughput, since typically the raw sample volumes are large and concentrations low. On the other hand, novel microfluidic chips for bacterial analysis are under development. This study solves the problems of the other methods by developing an automated bacterial filtering system using polydimethylsiloxane (PDMS) made cartridges. The system connects both meso-scale and micro-scale applications by providing an interface between milliliter volumes of sample and microfluidic chips requiring samples in microliter volumes.

Section II of this paper describes the design and fabrication process of the system. **Section** III discusses the test environment setup where the use of the system is demonstrated and **Section** IV describes the filtering sequence. **Section** V and **Section** VI explain the experiments with bacterial samples and their results, respectively. Finally, **Section** VII encompasses the conclusions.

II. DESIGN AND FABRICATION

Fig. 1 illustrates the different parts of the Automated Bacterial Filtering System (ABFS) with embedded microfluidic PCR chips. The main body of the automated bacterial filtering system consists of four chambers as follows: Diluted Sample Chamber (DSC) (1), Eluted Sample Chamber (ESC) (2), Elution Liquid Chamber (ELC) (3) and Filtrate Chamber (FC) (4). In addition to these chambers, there are one filter slot (5), two photointerrupter sensors (6) and (7), four microfluidic PCR chips (8) and finally eight channels which connect the chambers and the filter together.

A. Filtering Procedure

The proposed system has a simple and effective filtering procedure. A mixture of the sample and diluter liquid, diluted sample, 11 (ml), passes from the DSC (1) through the filter (5); the bacteria trap on the filter and the rest of the diluted sample is collected in the FC (4). The first photointerrupter sensor (7) detects the rear meniscus and assures that all diluted sample passes through the filter. Next the elution liquid, 1 (ml), is driven from the ELC (3) through the filter collecting the bacteria and carrying them to the microfluidic PCR chips (8) and the rest will fill the ESC (2). The second photointerrupter sensor (6) makes sure that microfluidic PCR chips and the ESC are totally filled with the eluted sample. Fig. 1 and Table I illustrate the filtering procedure. The numbering in Fig. 1 is explained in Table I.

B. CONCEPTUAL DESIGN

The main goals of the conceptual design were to minimize the number of required parts in the ABFS cartridges and at

TABLE I

COMPONENTS NUMBERS (BASED ON FIG. 1) AND VOLUMES OF ABFS.

	Name	Volume	Comp. No.
Chambers	Diluted Sample Chamber (DSC) Eluted Sample Chamber (ESC) Elution Liquid Chamber (ELC) Filtrate Chamber (FC)	11 (ml) 1 (ml) 1 (ml) 11 (ml)	No.1 No.2 No.3 No.4
Filter	VWR™Filter (Ø25 (mm), 0,2 (µm), PES membrane, 28145-501)		No.5
Sensors	Photointerrupter™ (SHARP®)		No.6,7
Chips	PCR Test Chips		No.8

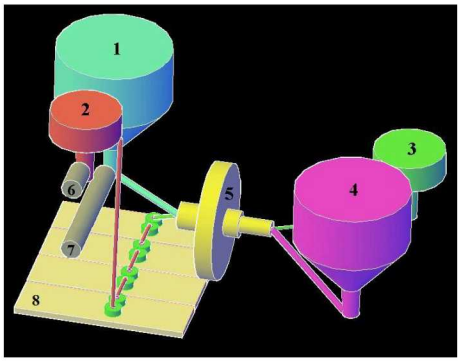

Fig. 1. Conceptual Design of Automated Bacterial Filtering System.

the same time to make a modular design which is usable for a large variety of filters for different applications. Using standard filter connectors in the mould design, makes this system modular to embed any standard filter with an arbitrary porosity size depending on the sample type. To achieve these goals, DSC, ESC, photointerrupters, PCR chips and a standard filter inlet were embedded in one cartridge; and ELC, FC and a standard filter outlet in another one. With this approach, the total required parts of the ABFS are two cartridges and one filter in between. Fig. 2 illustrates the 3D mould design for ABFS cartridges.

C. FABRICATION USING PDMS

PDMS is biocompatible, easy to cast and transparent which makes it a suitable material for ABFS cartridges. The mould for the PDMS (Sylgard 184 from Dow Corning) modules was done manually, building it from plastic pieces. Before casting, the PDMS base and curing agent were mixed with the ratio of 10:1, respectively. To avoid trapped air-bubbles in the large PDMS structure, the mould was casted gradually in three stages and vacuum was used between each stage. After curing for 4 hours in 60 (°C), the PDMS modules were demoulded.

III. TEST ENVIRONMENT SETUP

The test environment setup includes six main components; ABFS, HDI 3-Way Solenoid Valves (LEE Co.), Pressure Sensor (SensorTechnics Co.), Measurement Board (National Instrument®PCI6229), an in-house developed Pressure Generator Unit (PGU) and a computer with LabView interface.

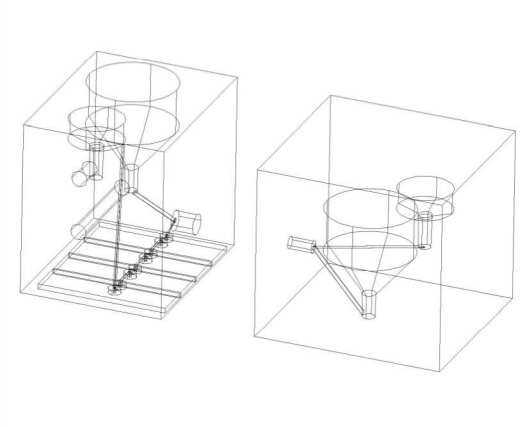

Fig. 2. 3D Mould Design for ABFS Cartridges.
Dimensions: Left: 60x48x63 (mm^3), Right: 57x57x50 (mm^3).

Fig. 3 illustrates the test environment setup. The three solenoid valves can connect the chambers to atmospheric pressure or to the PGU based on the defined filtering sequence. They are connected to the chambers using silicone tubes. FC and ESC are connected to the normally-open and normally-closed ports of Solenoid Valve 1, respectively. DSC and ELC are both connected to the normally-closed ports of Solenoid Valve 2 and Solenoid Valve 3, respectively. The pressure sensor is placed in line between the PGU and Solenoid Valve 1, and connected to an analog input channel of the measurement board. Measurement board is connected to the computer and interfaced using LabView. The PGU can generate pressures in the range of 1 (Bar) to -180 (mBar). Controlling the input voltage of PGU makes it possible to control the output pressure. Only negative pressures have

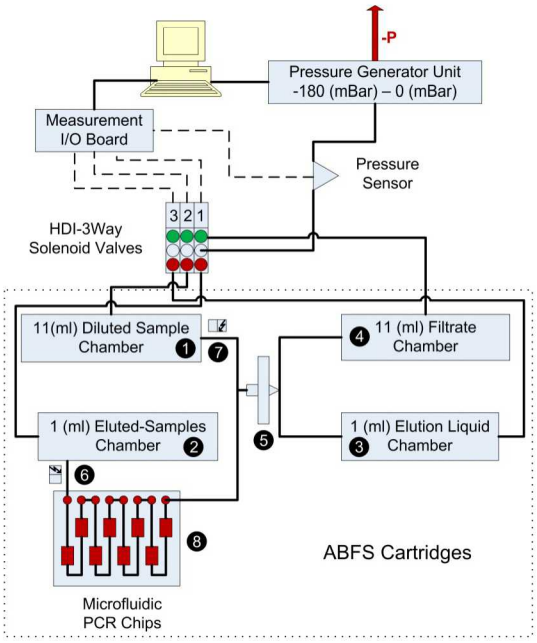

Fig. 3. Test Bench Scheme.

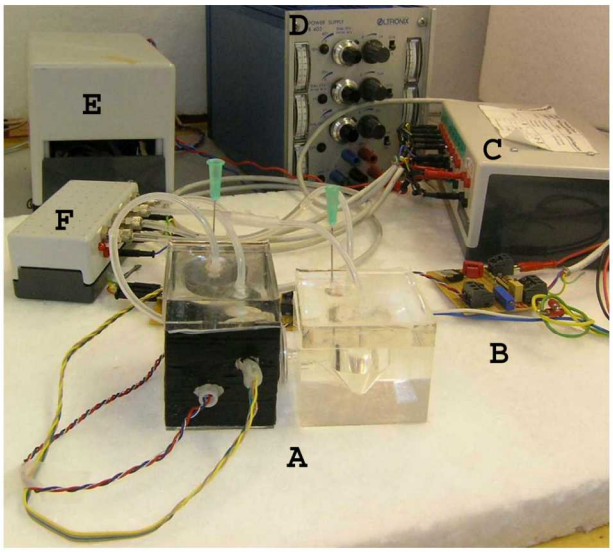

Fig. 4. The ABFS Test Bench. A) ABFS Cartridges, B) Photointerrupter Circuit Board, C) Measurement I/O Board, D) Power Supply, E) PGU, F) HDI-3Way Solenoid Valves.

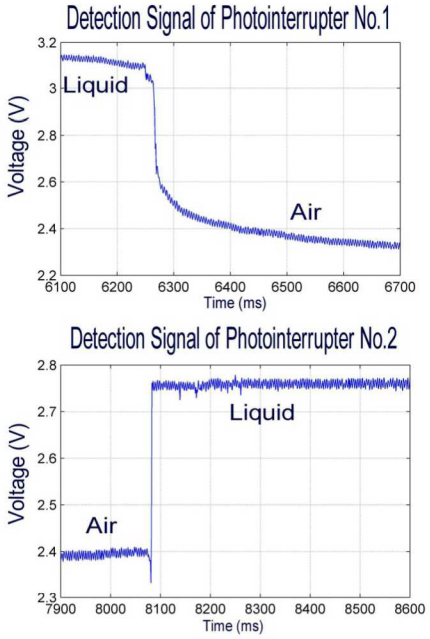

Fig. 5. Voltage Signals of Photointerrupters in Detection Phase.

been used in the ABFS to minimize the risk of leakage of hazardous bacteria out of the system. Photointerrupters are used to detect edges of the liquid flow: the rear meniscus of the diluted sample and the front meniscus of the eluted sample, in the channels. Photointerrupters are to guarantee the optimized filtering time. The ABFS was placed inside an opaque box to minimize the noise of the environment light. Fig. 4 shows the actual ABFS test bench.

The LabView interface triggers the valves based on photointerrupter detection signals and predefined time intervals. Fig. 5 illustrates the voltage signals of the photointerrupters in the meniscus detection phase.

IV. FILTERING SEQUENCE

The main advantage of the ABFS is its automatic sequence which makes it different from current bacterial filtering systems. The only semiautomatic part of the sequence is the injection of the diluted sample and the elution liquid. This part could be automated simply by adding two syringe-pumps to the ABFS. The filtering sequence includes the following four steps: *injection*, *filtering*, *stabilizing* and *collecting*. The chamber numbers used in the following are based on the numbering of Fig. 1 and Fig. 3.

A. INJECTION

The sequence starts with 5 seconds of pause which gives the operator enough time to get ready for the diluted sample injection. Then there is 30 (s) to inject the diluted sample and 20 (s) to inject the elution liquid. While injecting the DSC (1) and the ELC (3) are in atmospheric pressure.

B. FILTERING

The filtering starts with applying 180 (mBar) of negative pressure to the FC. Then DSC (1) is open to the atmospheric pressure and the diluted sample passes through the filter membrane. After the Photointerrupter 1 (7) detects the rear meniscus of the diluted sample, applying the negative pressure is continued for 20 (s) to assure that there is not any diluted sample left in the channel.

C. STABILIZING

To avoid unnecessary liquid flow between the chambers, stabilization is inevitable. The negative pressure will be reduced to 18 (mBar) for 10 (s), to stabilize the pressure in all chambers. Then the DSC (1) and FC (4) will be closed using the solenoid valves; the ELC (3) and ESC (2) are open to atmospheric pressure and the pressure is kept on zero for another 10 (s).

D. COLLECTING

It is necessary to have a low speed flow while collecting the bacteria. Therefore, 18 (mBar) of negative pressure, is applied on the ESC (2) while the ELC (3) is open to the atmospheric pressure. This makes a sufficient low speed flow for 5 (s) to collect the bacteria. The collected bacteria pass through the PCR chips (8) in their way to the ESC (2). Next, negative pressure increases up to 90 (mBar), until the front meniscus of eluted sample reaches to the ESC (2), and Photointerrupter 2 (6) detects it. Finally, applied negative pressure continues for another 40 (s) to be sure that there is nothing left in the ELC (3).

V. TESTS WITH BACTERIAL SAMPLES

Two series of tests have been done with bacterial samples to validate the functionality of the ABFS. The first experiments have been done with growth suspensions of *Escherichia coli (E. coli)* bacterium as samples and the second

experiments have been done with *Chlamydia trachomatis* bacterium.

A. TESTS WITH ESCHERICHIA COLI SAMPLES

The initial number of bacteria in the sample was determined using a regular plating method. Since there was only one ABFS cartridge available, having a proper disinfection procedure was inevitable before the experiments. The disinfection procedure encompassed five steps:

1) Dipping the cartridge in ethanol EtOH (70%).
2) Injecting ethanol through the channels.
3) Dipping the cartridge in the water.
4) Injecting water through the channels.
5) Drying with air pressure.

After each test, the disinfected PDMS cartridges were inspected to check if any bacteria remained in the cartridge after disinfection. 10 (ml) of 0.9% NaCl was used instead of *E. coli* sample and 1 (ml) of 0.9% NaCl was used as an elution liquid to do the filtering sequence for disinfection inspection.

The tests with *E. coli* consisted of two main parts, running the filtering sequence [See Section IV] and plating the eluted bacteria and the filtrate. The aim of plating both the eluted bacteria and the filtrate was to count how many bacteria had been collected *(recovery percentage)*, and to check that if any bacteria passed through the filter and went to the FC.

For each test, 1 (ml) of bacterial suspension was diluted with 9 (ml) of 0.9% NaCl, and this solution filtrated. After inserting the solution into the DSC, the filtering sequence was run. The filtrate was led to the FC and the bacteria were trapped behind the filter. Thereafter, 1 (ml) of 0.9% NaCl was led from the ELC through the filter into the ESC collecting the trapped bacteria. The eluate was collected from the chamber, the recovered volume was checked and the solution was diluted in 0.9% NaCl (10^{-2} - 10^{-5}) for plating to count the recovered viable bacteria. A 100 (µl) sample from the FC was plated without further dilution to assess the number of the bacteria which passed through the filter.

After the automatic tests, a few manual tests were done to make a comparison between the automatic and the manual recovery percentages. The manual tests were done using 10 (ml) regular syringe. The pressure generated with a syringe in the manual method is over +2 (Bar) which is 11 times more than the absolute value of the pressure used in the automatic method which was -180 (mBar).

B. TESTS WITH CHLAMYDIA TRACHOMATIS SAMPLES

Chlamydia trachomatis bacterial suspensions were grown and they were stored frozen at -70 °C. Bacterial samples were thawn and diluted 1:100 in 0.9 % NaCl. The number of the bacteria in the original suspension was not known. The dilution selected was based on an experienced estimation to contain an adequate number of bacteria. In this test 1 (ml) of prepared sample was diluted with 9 (ml) of 0.9 %NaCl, as the final sample; and 0.5 (ml) of elution liquid was used. The eluted sample was collected on the PCR chip illustrated in Fig. 6. After accomplishing the filtration process, the PCR chip was placed in the PCR machine to check the results of the filtering process.

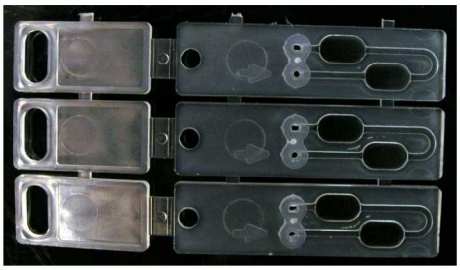

Fig. 6. PCR Microfluidic Chips.

VI. TEST RESULTS

The results of tests with *E. coli* are shown in Table II. An average of recovery percentage (ARP) using ABFS is 40.7% and in the manual method it is 67.3%. This difference in the ARP is mainly because of the difference in the applied pressure of these two methods which is 11 times more in the manual method than in the automatic method. Even though the ARP of the automatic method is less than of the manual method, it is sufficient for bacterial analysis. Furthermore, the ABFS is able to save a lot of time in frequent bacterial filtering applications. The average time required to run the automatic sequence is 210 (s) while it is 300 (s) for the manual method. This means that the ABFS saves 30% of the required time in each filtering sequence; besides it also minimizes the human interaction with bacteria and eliminates human error in the process.

The PCR machine showed a strong positive signal of *Chlamydia trachomatis* which proved that there were bacteria in the PCR chips. The number of bacteria in the PCR chip is not known, since the procedure is working based on amplifying the DNA of bacteria and not counting the number of them.

VII. CONCLUSION

In this case study, a prototype of an automated bacterial filtering system for interfacing microfluidic chips with a meso-scale sample volume has been designed and fabricated. Use of the growth medium sample was a crucial step in the development of an automated bacterial purification method.

TABLE II

THE RESULTS OF TESTS WITH *E. coli*.

Tests	No.	N.C.	A.F.S.	R.P.	A.R.P.
Automatic	I	1.3×10^6	4.9×10^5	37.3	
	II	1.3×10^6	6.8×10^5	52.6	
	III	1.3×10^6	4.2×10^5	32.3	40.7
Manual	I	1.3×10^6	8.3×10^5	63.6	
	II	1.3×10^6	9.2×10^5	71.1	67.3

N.C.: Number of Cells (cells)
A.F.S.: Average of Filtered Samples (cells)
R.P.: Recovery Percentage
A.R.P.: Average of Recovery Percentage

The feasibility, technical performance and flaws were found using pure samples which enabled fairly accurate quantification of inserted and collected bacteria. Modifications related to various sample matrices and their processing are being investigated. The system attained acceptable throughput during the tests. Compatibility of the system with frequent filtering processes, the disposability of filters and the implementation of commercial filters which cover a high range of biological samples make the system suitable for frequent laboratory purposes.

To be feasible for PCR purposes, cell lysis and the release of the nucleic acid contents must be considered. Isotonic elution solution was used in the presented ABFS in order to release the bacteria from the filter in viable form. This was a prerequisite for plate counting. In the intended system, cell lysis is to be performed by osmotic shock using a hypotonic solution for the elution, e.g. pure water. Hypotonicity induces swelling and burst of the cells thus releasing nucleic acid contents for the PCR and the heating in the PCR should complete the cell lysis. The principle of this approach, an osmotic shock combined with heating, has been shown earlier [8].

Few changes can be done to improve the performance of the ABFS.

The ABFS showed a slightly lower yield than manual filtering despite that the filters were identical in both systems. The lower recovery percentage can be due to a lower pressure applied in the ABFS, remaining of cells in the channels or some other reason, but rationally, modifying the PGU to apply higher negative pressures will increase the yield of the ABFS.

Selection of a filter may slightly contribute to a higher yield. Furthermore, there exist a multitude of filters featuring differences in porosity and hydrophilicity. A thorough comparison and selection of the filters was not seen necessary for pure growth medium samples used in the model system. Various samples and microbes behave differently and they may require specific filters and schemes for optimal performance.

Making the ABFS cartridges using hard plastic by injection molding instead of PDMS in the future make the cartridges sufficiently cheap to be disposable in addition to the filters. This can increase the safety of the tests and also eliminate the disinfection process.

Using two syringe pumps to inject the diluted sample and the elution liquid to the system can make the ABFS fully automated. A pre-filtering stage can be integrated to the ABFS to minimize possible clog up problems of the filter.

VIII. Acknowledgment

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