

A microfabricated polymer filter device for bio-applications

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ABSTRACT

The need for disposable diagnostic sensors in the health care industry has been a major driver in the development of low-cost polymer microfluidic devices. Of crucial importance to many of these devices is the incorporation of sieves and filters for the pretreatment of biological samples. Much of the previous work on integrating filtration systems in microdevices has focused on silicon and glass technologies. Of more difficulty, due to the different manufacturing methodology and lower mechanical strength, is the integration of filtration systems in polymer microfluidic chips. This paper presents a design and construction methodology to fabricate such integrated devices in polyethylene terephthalate (PET) and describes their characterization for particle filtration.

To demonstrate the application of these systems, DNA extraction from whole blood was investigated. This currently represents a major stumbling block for point-of-care diagnostics. To this end two approaches were taken; the isolation of leucocytes for subsequent DNA extraction, and the trapping of silica microspheres for DNA adsorption. The polymer surfaces of the fluidic chips were modified by UV exposure and chemical etching to increase their surface energy for improved non-specific binding and electroosmotic flow characteristics.

Integrated filtration devices were successfully fabricated with excimer laser machined membranes having pore dimensions down to 1µm, and contact angles from 75° down to less than 25° were achieved using UV modification, and from 75° down to 16° by chemical modification of PET. White blood cells were filtered from whole blood and silica particle retention was demonstrated successfully.

1. INTRODUCTION

With the promise of faster and more cost effective diagnostic devices the trend over the past couple of decades has been towards the miniaturization of laboratory-based protocols using microfluidic techniques^{1,2}. Microfluidic technology can offer not only cost savings and performance improvements but also give rise to applications in point of care testing through higher levels of integration and automation. These advantages have driven research in high throughput molecular diagnostics, advancing the fields of microarray and capillary electrophoresis^{3,4}. Miniaturised polymerase chain reaction (PCR) DNA amplification methods have also been developed and integrated with these detection systems⁵⁻⁸. These techniques, however, require relatively pure samples free from the many inhibitors that are present in many biological samples, such as haemoglobin, and some proteins and enzymes.

Polymer substrates have been investigated over the traditionally preferred materials of silicon and glass due to the need for low cost mass production. Most of the work to date has been on the development of batch-based processes for microfluidic chip construction. Of great potential for high throughput manufacturing are techniques that can be integrated into a continuous system such as reel-to-reel or web based processes. These web based systems are commonly employed in the packaging and printing industries where the structuring, lamination, printing, surface modification and bonding of plastics are routinely performed. However these technologies have been developed for other applications where the needs are far different from microfluidic requirements. Microfabrication processes need to be developed that are compatible with these continuous manufacturing systems. Multilayer film lamination has been demonstrated before with die cutting and CO₂ laser structuring⁹, however these techniques still provide relatively large-

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scale features, greater than 100 μm . The work presented here provides two alternative structuring methods; a direct write Nd:YAG laser that can provide cut widths down to 27 μm , and a photolithographic technique using an excimer laser that is limited to a resolution of 0.8 μm . These structuring methods are used to construct a device capable of extracting DNA from whole blood.

There are many commercially available kits used to purify DNA from biological matrices that are typically used in the laboratory, however these procedures are quite involved and are either performed manually or by expensive robotic systems. The two approaches for miniaturization that have gained some interest recently are blood filtration, where the leukocytes are isolated from the other blood constituents before they are lysed and the DNA is extracted, and solid phase extraction, or microparticle separation, which exploits the interaction of a silica surface to which DNA will bind preferentially under chaotropic conditions and can subsequently be eluted with water.

Microparticles have been used in laboratory-based protocols for a variety of applications, particularly in the areas of biology and healthcare where a large array of different particles, affinity mechanisms, and processes have been developed for techniques including immunoassay, cell separation and molecular biology. The difficulty in incorporating these microparticles into microfluidic systems is the method used to trap the spheres inside the channels. Microsphere retention for biological reactions has been demonstrated in silicon¹⁰⁻¹² and glass¹³⁻¹⁵. Of more difficulty is the creation of a solid phase system in polymeric devices where the structural strength is lower, compliance is greater, and fabrication methods differ.

Blood filtration has the advantage of not requiring these spheres, and therefore does not suffer from the difficulties in loading the spheres or from the high back pressures they generate. However the cells are highly deformable and will pass through restrictions much smaller than their diameter and can be broken by the shear forces generated in the filtration process.

The work presented here provides a method for integrating a filter into a microfluidic platform using lamination based technologies, particularly suitable for web-based processing such as used in the packaging and printing industries¹⁶. The methods of laser structuring, surface modification, and bonding are used to construct the filtration device, which is then used to demonstrate silica particle trapping and leukocyte retention.

2. EXPERIMENTAL

2.1 Fabrication

The devices were fabricated using PET film with a multilayer technique, chosen for its compatibility with web-based plastic film lamination systems for large-scale low cost manufacturing¹⁶.

Channel Formation. The PET film was cut using a frequency tripled Nd:YAG laser (AVIA 3.0W, Coherent Inc, USA) incorporated in a computer controlled 2-D laser cutting system (Lasertec, The Netherlands). The Q-switched, pulsed output of the laser generates a pulse duration of <30ns, a pulse energy of ~180 μJ , and for the purpose of this work a pulse repetition frequency of 10kHz was used. An f-theta lens having a focal length of 160mm was used to focus the beam to 30 μm in diameter at the work piece. Computer Aided design (CAD) drawings were used to control the temperature compensated, x-y, galvo-scanner and synchronise the firing of the laser. The beam velocity at the work piece is determined by the scanner parameters, and was set to 0.11 m/s. This combination of parameters results in a pulse being fired every 11 μm , or a shot overlap equivalent to 2.7 shots per area.

The devices were constructed from layers of Luminar S10 (Toray Industries, Inc.) PET film. Individual layers were cut entirely through by passing over the same tool path many times. Cleaning of the samples was performed by sonication in a 1:1 mixture of ethanol and water followed by multiple rinses in isopropanol and deionised water.

Membrane Fabrication. An Exitech S8000 excimer laser system equipped with a Lambda Physik LPX210I laser source was used to perforate the 12 μm PET film using standard mask projection lithography. The system illuminated a chrome-on-quartz mask with a homogenized 10mm square beam having an intensity deviation of approximately 5% RMS. A 10x projection lens with a NA of 0.3 was used giving a diffraction limited resolution of 0.8 μm .

Surface Modification. Chemical and UV surface modification were performed on both the 100 μ m and 12 μ m thick samples. The chemical treatment was achieved by placing the samples in a solution of 5M NaOH (Merck, Australia) at 80°C for 1min to etch, as previously reported¹⁷, followed by rinsing under flowing isopropanol then deionised water. The UV exposure was performed by placing the samples in a custom made light box with four 14watt metal halide lamps (Amber Lamps, GPH369T5VH). The samples were placed 60mm directly below the lamps and measurements were taken for timed exposures of 10, 30, 60, 150, 360 mins.

Bonding. An in-house embossing system was used to thermally bond the substrates. The unit consisted of a regulated hydraulic press (REXROTH, Australia) that applies pressure to the embossing chamber, with the temperature controlled (SHINKO, Australia) heating elements and forced air cooling. The samples were sandwiched in-between 1mm of paper and flat plates of steel to ensure even pressure distribution. The chamber was evacuated (-80kPa) and preheated to 190°C before being compressed to 7MPa for a period of 45 mins.

2.2 Characterisation

Cut profile characterisation and sample visualization was performed with a BX60 Olympus optical microscope. Detailed images were taken on a JEOL, JSM35, scanning electron microscope using gold coated samples, prepared with a Polarin Equipment sputtering unit. Height measurements were made using the Olympus OLS1200 He:Ne laser scanning confocal microscope.

Surface characterization was performed using XPS and contact angle measurements using a goniometer (Ramehart, Inc). The samples were prepared by sonication in a 1:1 mixture of ethanol and water followed by multiple rinses in isopropanol and deionised water. Samples were stored in air and analysed by XPS within two days. The XPS data was collected on a Kratos AXIS Ultra imaging XPS instrument using a monochromated Al K α X-ray source at a power of 1486.6 eV. The energy scale of the instrument was calibrated using the Au 4f7/2 photoelectron peak at binding energy (EB) = 83.98 eV. Charge correction was performed using the C 1s photoelectron component peak corresponding to C-C species at EB = 284.7 eV. As the samples were insulating, charge neutralisation was performed using low energy electron bombardment.

2.3 Fluidic Performance

Flow and leakage tests were performed using custom computer controlled dual syringe pumps and pressure sensors. Flow rates from 10 μ l/min to 1ml/min were used with back pressures up to 50psi. The 5 μ m silica particles used for packing the channel were purchased from Bangs Laboratories. Whole peripheral blood was collected by finger prick of a healthy Caucasian male, and solutions of methylene blue, triton x and phosphate buffer were all prepared in deionised water.

The current monitoring technique developed by Huang¹⁸ was used to measure the electroosmotic flow. This was achieved by filling a single channel with 25mM Phosphate buffer, emptying the reservoir at one end and replacing it with a 50mM solution of the same buffer. A HP5r high voltage supply (Applied Kilovolts, UK) applied a potential of 200V/cm across the reservoirs, while the current was monitored across a 10k Ohm resistor between one of the reservoirs and ground using a AT-MIO-16E-10 (National Instruments) data acquisition card. The current was monitored until it reached a plateau giving the time taken for the channel to fill with the less concentrated solution, dividing this by the length of the channel gave the electroosmotic velocity.

3. RESULTS & DISCUSSION

3.1 Fabrication

The filters were fabricated by excimer laser machining¹⁹ of 12 μ m PET film, with pore dimensions from 50 μ m down to 1 μ m. Fig. 1 shows a hole array of 10 μ m pitch with 8 μ m entrance and 1 μ m exit holes. For the filter to maintain a consistent pore size under increasing pressure the wall angles of the pores needed to be minimized to maintain good structural stability. The fluence versus wall angle for excimer ablation has been well characterized in the past²⁰ and it has been shown that the higher fluences produce steeper wall angles. To this end the laser was operated in constant energy mode with a fluence of 1.5 J/cm² at the workpiece, giving ablated wall angles of approximately 10 degrees.

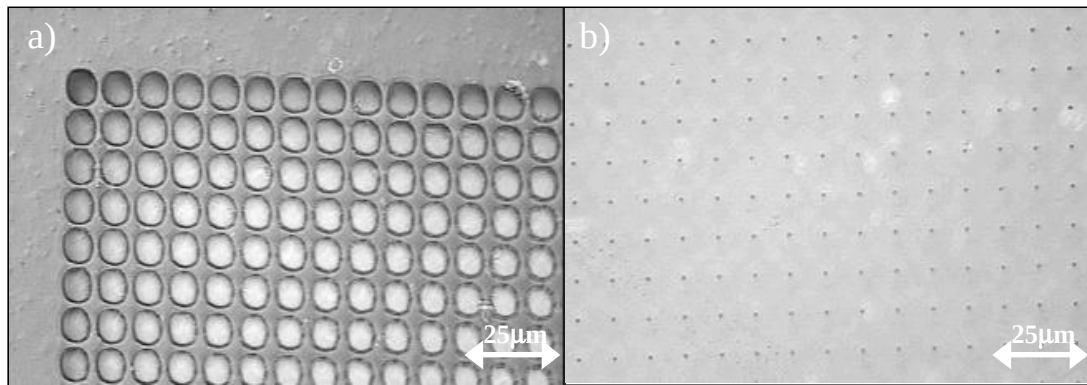


Figure 1: Excimer ablated filter membrane in 12μm PET a) Entrance holes 8μm b) exit holes 1μm

The device was patterned using the 355nm YAG laser operating with a beam diameter of approximately 30μm, with a focal depth less than 100μm. As reported in earlier work¹⁷ the cut width before bonding varied according to the substrate thickness, 100μm substrates gave a cut width of $40 \pm 5 \mu\text{m}$ whereas the 12μm film gave a much larger cut width ($120 \pm 20 \mu\text{m}$) unless a fluid, such as isopropanol, was used to help dissipate the heat, giving a cut width much closer to the beam's diameter ($27 \pm 2 \mu\text{m}$). The chip layers were then cleaned with isopropanol in an ultrasonic bath removing most of the smaller pieces of ejected material that had redeposited around the cut, leaving only the larger fragments behind, as shown in Figure 2a). The debris and ridges formed from the laser cutting process, which were as large as 20μm high, were evenly compressed back into the bulk giving steeper wall angles and a more even surface (Figure 2b)).

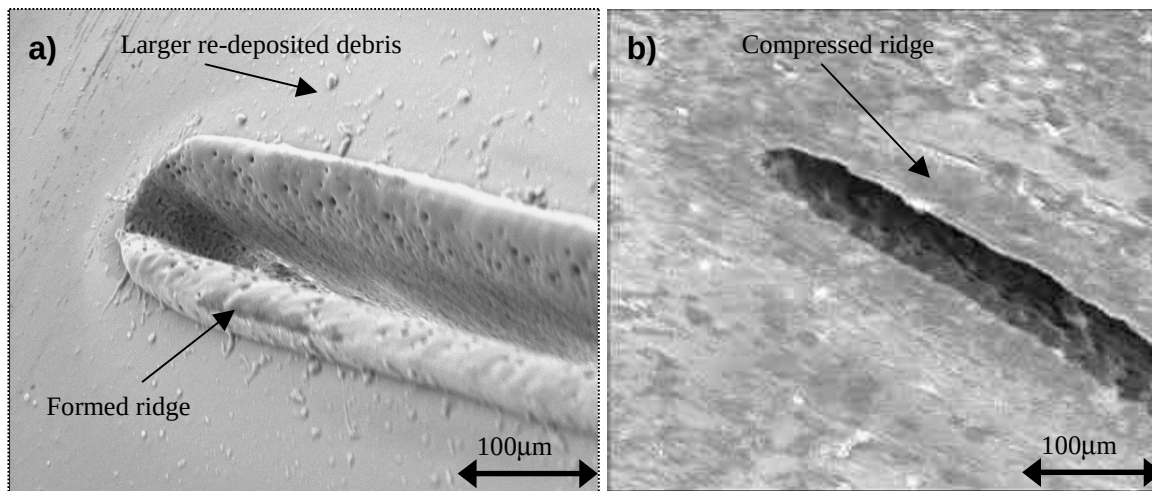


Fig. 2: Scanning electron microscope image of laser cut PET before a), and after b) bonding

The diffusion bonding method used to prototype these devices requires elevated temperature and pressure to allow the like substrates to diffuse into one another. Therefore when bonding a multilayer structure like this it was necessary to use a multistep bonding method to ensure that the membrane layer was fully sealed above the exit channel. In this case, layers 3 to 5 were firstly bonded together before layers 1 and 2 were added. Figure 3 a) shows the product of using a single step bond and b) the result of a good bond from a multistep procedure. The rough edges seen at the ends of the channel are artifacts from using the laser in the cross sectioning process.

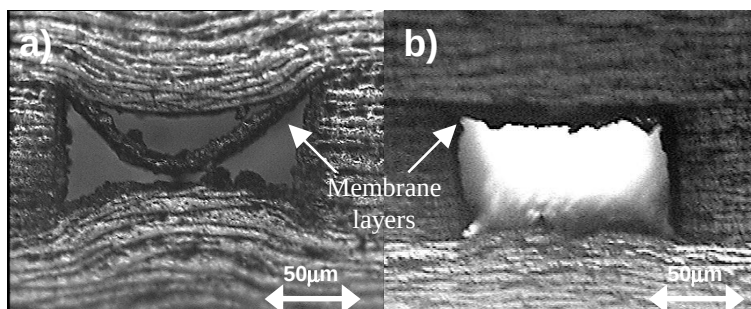


Figure 3 Channel cross section of a) single step bonding, and b) two step bonding procedure

As shown in previous work¹⁷ the cutting process increases the hydrocarbon component on the surface, decreasing the surface charge. In some applications this increase in hydrophobicity can aid in electrophoretic separations by reducing the electroosmotic flow and thereby helping to retain the sieving matrix and increase the separations. However, protein non-specific binding is increased with the more hydrophobic surface, and capillary action effects are also reduced making it difficult to fill micron sized channels with aqueous solutions²¹. Therefore it is desirable in many biochip applications to increase the surface charge and hence decrease the polymer hydrophobicity. These charges can also be reacted with an amine, such as *N*-methyl-1,3-propane diamine²², to form amine terminated groups that can undergo further reaction to bind oligonucleotides to the surface for DNA hybridisation based experiments²³.

To increase the surface energy two techniques of surface modification were investigated; saponification or base hydrolysis¹⁷, and UV exposure, as has been demonstrated on polycarbonate by Liu et al²¹. The base hydrolysis was found to have an optimal exposure time after 1 min and gave a contact angle reduced from 75° to 16°, whereas the UV modification required 1hr to give a contact angle down to 25°. Furthermore the UV contact angle increased to around 35° upon washing, which may be attributed to the removal of low molecular weight fragments.

XPS surface analysis was performed on standard PET and modified PET samples with exposure times of 1min for NaOH modified and 60mins for UV modified. Table 1 shows their relative percentage atomic concentrations, showing there is a significant reduction in the level of carbon and an increase in the oxygen concentration for both treated samples. As expected from the lower contact angle the chemically modified sample has significantly higher oxygen content in addition to a large amount of sodium contamination due to residual NaOH.

Table 1: Relative % atomic concentration of the carbon, oxygen and sodium elements present on the surface of base hydrolysed, UV modified and clean PET as determined by XPS.

Sample	Relative % Atomic Concentration		
	O	C	Na
Clean PET	26.8	73.2	-
UV Treated PET	32.9	67.1	-
NaOH treated PET	37.5	45.1	16.4

3.2 Fluidic Performance

A simple in-line filter design was used to demonstrate particle filtering using a multilayer laminate construction. The device fabricated, shown in figure 4b), provides multiple test structures, two of which have dual inlets for reagent delivery, an extended channel to allow for greater time for diffusive mixing, and a large filter chamber for particulate retention. The third structure provides two intersecting channels via a long narrow filter region with the same porosity as the other test structures. The device construction, as outlined in Fig 4a), consists of 5 layers of PET film; a bottom sealing layer, exit channel layer, the filter layer, the inlet channel layer, and a top-sealing layer with entrance and exit ports. Figure 4 b) shows an assembled device with a close-up of the filter chamber.

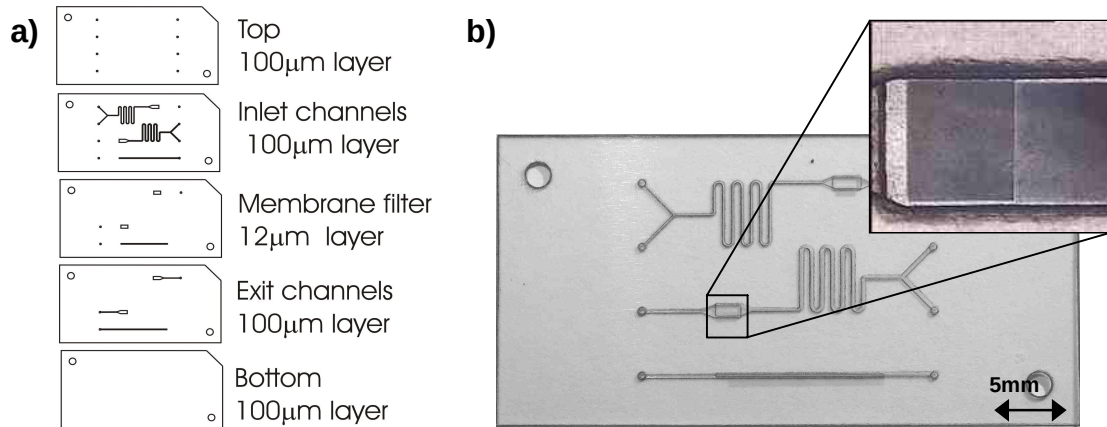


Figure 4 Layered approach to microfluidic chip construction a), and fabricated device with insert showing filter chamber b)

To maximize flux for a given pore size the pitch needed to be minimized without reducing the membrane's strength to the point of failure. The filters were designed with $4\mu\text{m}$ exit holes at a pitch of $10\mu\text{m}$ giving rise to an effective porosity of approximately 13%. Reducing the pitch further resulted in the inlet of the pores overlapping, thinning the membrane and decreasing its strength greatly.

The filter's surface energy plays an important role when initially filling the device or when air bubbles are introduced into the system. The pressure required to initially pass the liquid through the membrane is the sum of the applied pressure P_a and the effective pressure introduced from the surface tension, P_s ²⁴.

$$P_s = \gamma \cos \theta / r \quad \text{where, } \gamma = \text{surface tension} \\ \theta = \text{contact angle of the fluid with the surface} \\ r = \text{pore radius}$$

If the membrane is more hydrophilic then $\cos \theta$ approaches one and small pores like those used here will fill by capillary pressure. For surfaces with contact angles greater than zero an applied pressure is required to pass fluid through the pores. The membranes fabricated in unmodified PET with pore dimensions of $4\mu\text{m}$ gave a bubble pressure of 1.8 ± 0.16 psi, which is below the calculated value of 9.8 psi. This may be attributed to the combination of factors from the pore exits having slightly rounded geometries and varying in size, a variation of surface tension, and the bubble point pressure method being limited to measuring the largest pore hole present.

The electroosmotic flow velocities were measured for the untreated and chemically modified samples. In both cases the direction of flow was from the anode to the cathode, confirming the presence of negatively charged surface groups. The unmodified PET gave an electroosmotic mobility of $0.76 \times 10^{-4} \pm 0.12 \times 10^{-4} \text{ cm}^2/\text{Vs}$ and after a 5 min NaOH treatment this was increased to $1.2 \times 10^{-4} \pm 0.14 \times 10^{-4} \text{ cm}^2/\text{Vs}$. These values indicate that YAG laser cut channels in unmodified PET give a comparable result to embossed polycarbonate, as demonstrated by Liu et al who achieved mobilities of $0.7 \times 10^{-4} \text{ cm}^2/\text{Vs}$ ²¹. Likewise, the 3 hour UV treatment used by Liu et al produced a similar result to the 5min base hydrolysis treatment with an increase in mobility to approx $1.25 \times 10^{-4} \text{ cm}^2/\text{Vs}$.

Bead Trapping. Silica microspheres were introduced by pipetting an aqueous solution of 10% beads and 1% Triton X into the device and applying a vacuum (10psi) to the channel outlet. Under this vacuum the beads took approximately 30mins to pack the column tightly, leaving only a small trailing amount of loosely packed particles, as can be seen in Figure 6a) which shows a $225\mu\text{m} \times 100\mu\text{m}$ packed channel. Upon introduction the beads immediately filled the length of the channel covering the filter, increasing the backpressure and reducing the flow rate. The image in figure 6b), of a channel with the top layer removed, clearly shows the end of a column packed with the microspheres. Increasing the applied pressure speeds the packing process, however when using $5\mu\text{m}$ beads with only a $4\mu\text{m}$ porous filter then greater forces cause the microspheres to deform the membrane and pass through the filter. This was not a problem for larger bead to pore ratios.

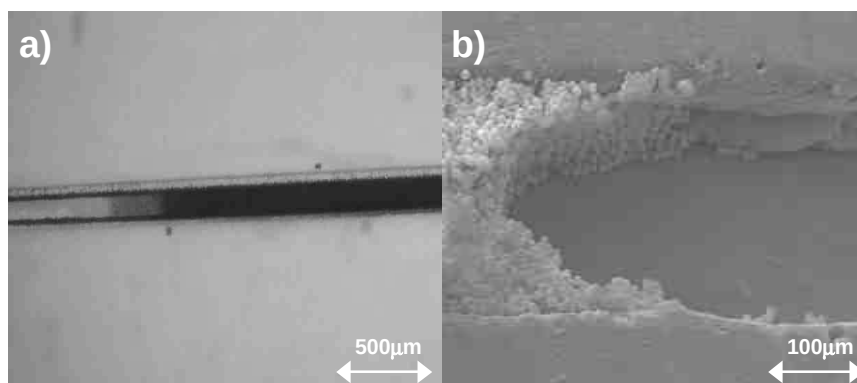


Figure 5 Silica particle retention a) tightly packed column, b) image of microspheres in a channel with the capping layer removed.

White Blood Cell Filtering. The difficulty with whole blood filtration lies within the elasticity and structural strength of the biological cells. The Leukocytes, or white blood cells (WBC), we are isolating from peripheral human blood are highly deformable and range in diameter from 6-8 μm for the smaller lymphocytes to 12-20 μm for the monocytes, with the most abundant being the neutrophils that comprise approximately 60-75% of the WBC present and are around 10-15 μm in size. Key to the successful isolation of DNA for PCR is the removal of the red blood cells containing hemoglobin that inhibits the amplification process. Red blood cells are a relatively rigid discoidal shape 7 μm in diameter and 2 μm thick. Blood filtration in silicon based devices has been demonstrated by Wilding et al¹² who used weir and pillar filters, at a flow rate of 0.035 $\mu\text{l}/\text{min}$, and found that the red blood cells easily pass through gaps as small as 3 μm , whereas the much larger and highly deformable white blood cells can pass through gaps as small as 7 μm .

A mix of whole blood 2 μl , in 20 μl of 0.005% methylene blue (for nucleus staining) was injected into the device followed by a wash with 200 μl PBS. The clear red blood cells and smaller stained white blood cells passed easily through the membrane with 4 μm pores, leaving a cake of mostly stained leukocytes (Figure 7). With the filter clogged the flow rate was greatly reduced using the unmodified PET membrane, and back-flushing met with only limited success.

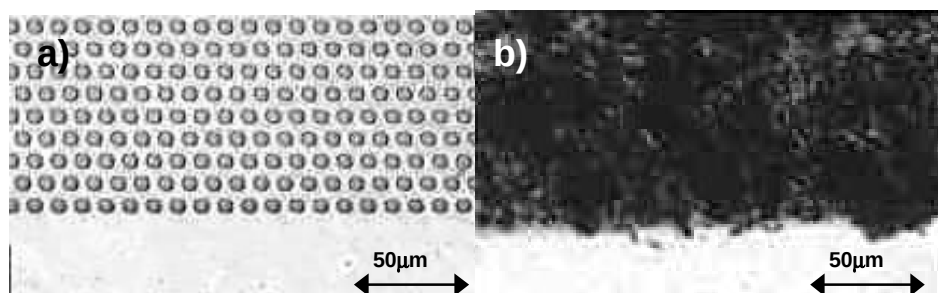


Figure 7. Membrane with 8 μm entrance pores a) before filtration, and b) after filtration of methylene blue stained white blood cells.

Modifying the membrane with the 1min chemical treatment before the device was assembled produced a much more hydrophilic membrane that had less irreversible binding of particles to its surface and successfully allowed back-flushing to occur.

4. CONCLUSION

Laminated microfluidic networks with integrated filtration have been successfully constructed in PET using Nd:YAG and excimer laser structuring. Microfluidic devices having channel dimensions down to 100x100 μm have been demonstrated with integrated filters having pore sizes as low as 1 μm . The surface modification techniques presented can reduce the contact angle from 75° to 16°, increasing electroosmotic mobility from $0.76 \times 10^{-4} \text{ cm}^2/\text{Vs}$ to 1.2×10^{-4}

cm²/Vs. Silica particle retention and white blood cell trapping have been developed as a first step towards a polymeric DNA extraction device. Although batch based diffusion bonding was used for this work, the layered chip construction method demonstrated is compatible with web-based manufacturing technologies, suitable for high volume low cost manufacturing.

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