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LETTERS

A novel Fusion-5 Filter based Micro-chip: A highly efficient, On-chip DNA extraction and On-chip PCR amplification for rapid detection

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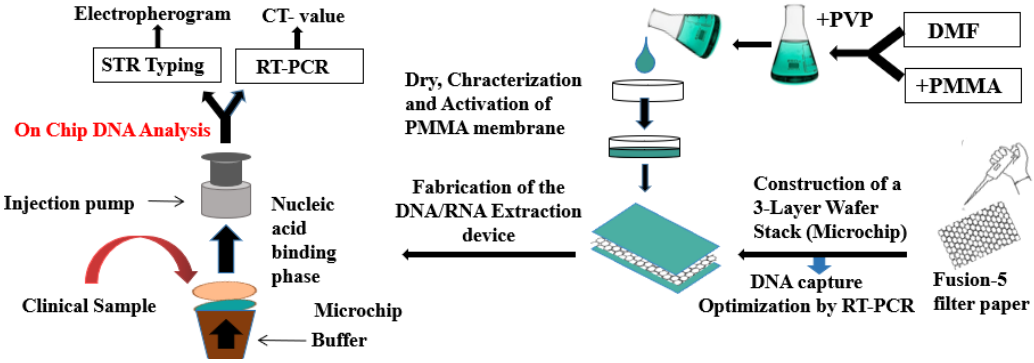
Article

ABSTRACT



A novel, sensitive, portable method for detecting DNA from various human samples is most desirable. This study aims to develop a microdevice for on-chip DNA extraction and detection by PCR from various forensic samples. A microchip constructed by sandwiching a fusion-5 filter disc between PMMA layers was characterized using SEM, FTIR, and XRD. DNA capture efficiency of the Microchip from human samples was quantified by Real-Time PCR. PCR products were evaluated off-chip by DNA sequencing (STR Typing). On-site detection was performed by visualizing the DNA amplicons on Fusion-5 filter paper under UV light after EtBr staining. Among all concentrations, 5% by weight PMMA membrane was found most suitable for PMMA-Fusion-5 filter disc fluidic Microchip, the best smooth cross-section morphology by SEM, strong absorption vibrations at corresponding wavelengths by FTIR, increased amorphous phase by XRD were confirmed. Using this microdevice, DNA extraction from human whole blood was, without any leakage, fast (≤7 minutes), most efficient (highest, Ct=27.22) as evaluated by real-time PCR, needs just 2µl blood sample as shown by a typical, balanced STR profile. The microdevice designed for on-chip DNA extraction has excellent potential for rapid DNA on-site detection from various samples.

Keywords: Fusion-5 filter, PMMA (Poly methyl methacrylate), On-chip DNA Extraction, DNA capture efficiency, On-chip PCR Amplification, Rapid detection

INTRODUCTION

The DNA analysis procedure at the forensic laboratory often takes days, resulting in the outcome becoming less relevant to effectively contribute to the initial stage of the criminal investigation conducted by the police forces.[1,2] It might give the perpetrator time to eliminate relevant evidence, elope, or even commit another crime. At the same time, it is desired that innocent suspects be released quickly from custody.[3] So, the first few hours of investigation are called the "golden hours," which is not without reason. For these reasons, there is a strong need for relevant information to become available as quickly as possible.[4] Technology or Devices that provide immediate information to police investigators at the crime scene are more beneficial, as direct analysis helps develop a fast and effective case scenario. A fully integrated instrument that will provide samples into the results of forensic DNA analysis will dramatically reduce the costs (including labor, space, and validation) of establishing and operating a forensic DNA lab. On-chip DNA extraction technology has been developed for this purpose. It is based on the capture mechanism of DNA adsorption on silica surfaces in the presence of high chaotropic salt concentration.[5] The first on-chip DNA extraction was demonstrated by Christelet al. on a micro device with silica pillars fabricated by deep reactive ion etching (DRIE) for nucleic acid capture.[1] Instead of microfabrication, Breadmore et al. from Landers' group successfully created a silica bead-packed column with a sol-gel matrix in a microdevice for DNA purification, demonstrating a nearly 80% DNA recovery rate.[6,7] Magnetic silica beads are retained by a magnetic field and are used as a fluidic capture microchannel for DNA isolation from blood. [8,9] While silica-based methods are effective, using PCR-unfriendly reagents, high-concentration chaotropic, and organic solvents often pose problems to downstream amplification. To circumvent these problems, Cao et al. utilized chitosan-coated

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beads to extract DNA at pH 5. They released it from the beads at pH 9, achieving an impressive extraction efficiency of 75% from whole blood samples.[10] Different surface modification methods, such as amine and carbonyl/carboxyl group grafting, were also proven effective for pH-induced DNA extractions. Unfortunately, the utilization of on-chip DNA extraction methods on a daily basis so far is still rare. The reason is that no single microdevice has satisfied all the criteria for optimal DNA extraction. An optimal DNA extraction microchip should fulfill the minimum requirements, such as providing adequate inhibitor-free DNA to downstream analyses, being inexpensive, automated, capable of processing various raw samples, and suitable for integrating downstream steps to form a total analytical system. Firstly, to lower the cost, various polymer substrates, such as poly (methyl methacrylate) (PMMA),[11–13] polycarbonates (PC),[14] and photo-activated polycarbonates (PPC),[15] have been employed in the fabrication of DNA extraction systems. More recently, a microdevice made up of polymer sheets and paper has been developed to enable truly low-cost, point-of-care sample preparations in the developing world.[16] These inexpensive materials not only simplify the manufacturing process but also eliminate the risk of cross-contamination by enabling one-time use of the devices. In this study, a Fusion-5 membrane disc sandwiched between polymer sheets was employed to capture the DNA, and a syringe pump was used for drawing samples through the disc integrated into a microfluidic device where the Fusion-5 filter disc acts as a solid capture phase for low-cost, rapid DNA extraction from whole blood samples. The DNA capture efficiency of the microdevice was examined by quantifying DNA extracted from human whole blood and various other raw samples and further off-chip PCR amplification following DNA detection on the device itself. Thus, to the best of our knowledge, our lab on chip is one of its kind, which not only extracts DNA on the spot but can also detect the same within a few minutes.

MATERIALS AND METHODS

Poly methyl methacrylate (PMMA) (m. t. >150º C, MW 996k by GPC and size 120-160 mesh), Dimethyl formamide (DMF), Polyvinyl pyrrolidone (PVP), and Amino propyl tri ethoxy silane (APTES) were procured from Sigma Aldrich Chemicals. Fusion-5 filter paper (size 2.3µm) and Whatman Grade FTA paper were purchased from GE Healthcare. DNA extraction reagents like 1% Phosphate buffer saline, 10 mM sodium hydroxide solution, 10 mM EDTA solution, 1 mM TRIS-HCL, and 5 mg/ml Ethidium Bromide (EtBr) solution were prepared using Sigma Aldrich molecular biology grade chemicals. Four different types of forensic samples, like human whole blood in EDTA vial, dried blood stains (DBS), buccal swabs, and saliva, were also procured from the forensic laboratory in Delhi and stored at 4 ºC until further use. The Quantifiler Duo DNA quantification kit for quantitation and amplification and AmpFiSTR Identifiler Plus PCR amp kit for STR typing of the DNA were purchased from Life Technologies, USA.

Instruments and Equipment

Scanning Electron Microscope (EVO-18, Carl Zeiss, Germany) was used to study the surface morphology of PMMA film and the prepared PMMA fused with fusion-5 filter paper. The X-ray Diffractometer (X'PERT PRO X-Ray Diffraction) recorded the XRD pattern of polymer thin films. Fourier transform infrared spectroscopy (FTIR, Bruker IFS 66 V/S) was used to study the details of functional groups. Real-time PCR analyses were performed on Applied Biosystems 7500 Fast Real-Time PCR System using the Applied Biosystems Quantifier Duos Kit software 1.2.[17] Real-time PCR is one of the recent and trusted technologies used to detect DNA or RNA in many diseases.[18] Veriti thermal cycler from Applied Biosystems was used to perform PCR. Optical detection of DNA was done using a UV Trans-illuminator. Applied Bio 3500 Series Genetic Analyzer was used for STR Typing using standard gel electrophoresis technique.

METHODOLOGY

Design of Microfluidic Device

The benchtop nucleic acid protocol can be adapted to a microfluidic format by preparing a microchip incorporated within a microdevice. A single-use, disposable chip was constructed using a three-layer wafer stack consisting of plastic substrates (from top to bottom), an upper PMMA layer, and a lower PMMA layer and in-between a 3 mm diameter piece of Fusion-5 filter paper sandwiched (Figure 1A).

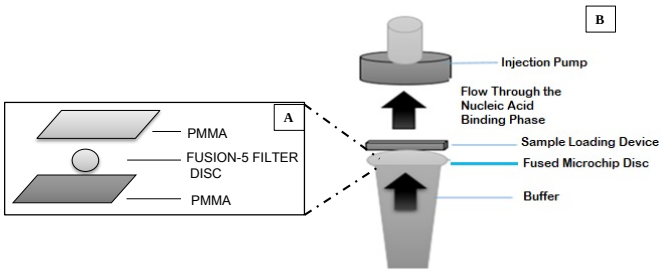


Figure 1. Schematic Design of Microfluidic Device: (A) The three-layer wafer stack, PMMA layers sandwiching layer a Fusion-5 filter disc. (B) Working Design of the Microfluidic device used for DNA extraction.

Preparation of PMMA Membranes

The solid polymer films of Poly methyl methacrylate (PMMA) were prepared using a solution casting technique with certain modifications. In a 250 ml flask, the quantity of PMMA was taken and dissolved in solvent Dimethyl formamide (DMF), heating to 80 ºC. Polyvinyl pyrrolidone (PVP) was added into the flask, and the mixture was stirred at a magnetic stirrer at 80 ºC for 4 hours until a homogenous solution was obtained. PMMA casting solution was poured onto the petri dish and dried overnight. PMMA at different concentrations, as shown in (Table 1) (2%, 3%, 5%, 6%, and 8% by (PMMA+PVP) weight), was taken to study the optimum thickness of the PMMA membrane to capture the maximum of DNA.

Membrane Characterization

Membrane characterization was performed by (i) Scanning electron microscopy, (ii) X-ray diffraction technique (XRD), and (iii) Fourier Transfer Infrared Spectroscopy (FTIR). SEM micrographs of pure PMMA blend films were taken using

Table 1. Concentrations of PMMA (by wt.) and PVP used to prepare polymer films.

S. no.	Amount of PMMA (gms)	Amount of DMF (ml)	Amount of PVP (gms)	D. Water (ml)	PMMA+PVP (by wt.)
1.	0.2	10	0.100	5	2%
2.	0.3	10	0.150	5	3%
3.	0.5	10	0.250	5	5%
4.	0.6	10	0.300	5	6%
5.	0.8	10	0.400	5	8%

Scanning electron microscopy. The top surface of the PMMA membrane was analyzed after coating the sample with a thin gold-platinum film. The XRD patterns of polymer films were recorded using an X-ray Diffractometer. The diffraction data were taken at room temperature with Bragg's angles (2ɵ) varying from 10 °C to 80 °C degrees. FTIR spectroscopy measurements of pure PMMA and Fused PMMA (PMMA + Fusion-5) were recorded with the help of a spectrophotometer.

Activation of PMMA Substrates

The surfaces of the PMMA substrates were activated for 120 sec with oxygen plasma (30% power) to generate hydroxyl groups. Then, the surfaces were silanized using a 2% aqueous solution of APTES (aminopropyltriethoxy silane) at 55 °C for 1 hour, followed by thoroughly washing with water and drying with N2.

Optimization of Filter Paper

The Fusion-5 filter paper, along with other filter papers, including Whatman Grade 1 of retention size (11µm), 2 (8µm), 3 (6µm), and 5 (2.5µm), was tested to be chosen as best binding phase materials. To test and compare the DNA capture efficiency, 10 ng of 9947A Standard (Amp FL STR Identifiler Plus) DNA with a concentration of 1 ng/µl was pipetted onto the surfaces of filter discs and washed once with 100µl of TE buffer to elute out the DNA captured on the disc. After that, the filter discs were taken for RT-PCR. To quantify DNA trapped in the filters, real-time PCR analysis was performed using Applied Biosystems Quantifiler Duos Kit software 1.2 on Applied Biosystems 7500 Fast Real-Time PCR Systems, containing total PCR master mix volume 23µl with 12.5µl quantifiler mix each, 10.5µl primer mix each loaded onto the filter disc. Then 2µl of the sample, standard or control, is added, making the total reaction volume 25µl. Comparison data on DNA capture efficiencies on all these filter papers was made by estimating concentrations of DNA per µl liberated and recording CT values by RT-PCR.

Construction of a 3-Layer Wafer Stack

To employ solid phase nucleic acid capture filter paper on a microfluidic system, the Microchip was designed consisting of three layers of wafer stack of PMMA-Fusion-5-PMMA layer (Figure 1A), which were aligned together and heated up to the bonding temperature of 95 ºC, before the bonding pressure was applied. Once the bonding temperature became constant, a bonding pressure of 4 bars was applied for 30 minutes. When the substrates cooled down to 75 ºC, bonding pressure was unloaded, and the sample was allowed to cool down at 50 ºC for 2 hours. A solid and irreversible PMMA–Fusion-5–PMMA bond was constructed using a novel in-house device, a DNA microchip fuser working on the principle of hydraulic compression press designed into a cabinet.

Fabrication of the Extraction Device

A plastic microfluidic device using a standard syringe pump was produced to operate the Microchip (Figure 1 B). The device fabrication was as follows: a plastic-made syringe was taken to provide a flow-through of the nucleic acid capture membrane at the inlet. A buffer column was connected at one end, along with a sample loading valve in the middle and a syringe pump at the inlet to provide fluidic connections. Both the pump and valves were controlled manually to facilitate the operation.

Sample Preparation

Human whole blood samples, buccal samples, saliva, and dried blood stains were obtained from the forensic science laboratory. Each biological sample was loaded onto the fused Microchip. For liquid samples like blood and saliva, 2µl was pipetted onto the microchip surface using a micro-pipette. For swab samples like buccal swabs, the swab head was pressed directly onto the chip using three side–to–side motions to deposit the sample. To analyze the dried blood stains, small pieces of blood stains were sliced off using a surgical blade, mixed with phosphate buffer saline, and vortexed to prepare a DBS-PBS mixture, and then 2µl of it was loaded onto the paper. All the samples were allowed to air dry for at least 3 hours before being placed in the DNA extraction chamber.

Working Principle

The sample work-up consists of three steps: cell lysis, DNA extraction, and DNA purification. The last two steps can be combined when performed on-chip. Once the lysis of the cells is completed, the DNA is separated from the other cellular components since these can inhibit the amplification reaction. Inhibitors generally present in forensic samples are ethanol and sodium-dodecyl-sulfate (SDS) chemicals from the isolation technique. Alongside hemoglobin, calcium-ions, melanin, and urea, possibly present in biological samples. These inhibitors can delay the threshold cycle, reduce the detection sensitivity (especially for larger amplicons), or even failure of PCR amplification. Therefore, to prevent the involvement of inhibitors, the alkaline method of isolation is taken up.

DNA extraction by Alkaline cell treatment method

The real-world samples, like whole human blood, saliva, buccal swabs, and dried blood stains, were loaded onto the paper and dried before being placed in the DNA extraction chamber in the microdevice to verify its extraction capabilities. The DNA extraction process begins with the washing step using 200µl of DI water aspirated through the disc by a syringe pump. After that, 100µl of a 10 mM sodium hydroxide solution (NaOH) along with 10 mM EDTA was drawn into the DNA

extraction chamber and incubated at room temperature for 5 minutes to lyse the cells completely, followed by adding 50µl of TRIS-HCL (1mM). It is a neutralization reaction. The final washing step is done using 50µl of DI.[19] All the reagents mentioned above were manually aspirated through the filter disc using the syringe pump. The whole process was finished in 7 minutes. Finally, the Microchip was disassembled, and the fused disc was removed for PCR quantification.

DNA extraction efficiency on Fusion-5 Filter paper

The efficiency of the DNA extraction on the Microchip from human whole blood and other samples was tested by performing DNA quantification on Applied Biosystems 7500 Fast Real-Time PCR System. DNA templates on Fusion-5 paper were quantitated using Applied Biosystems Quantifiler Duos Kit software 1.2 containing reaction loaded onto the disc of total PCR volume 23µl with 12.5µl quantifiler mix each and 10.5µl primer mix each. A calibration curve was generated using serially diluted standard DNA dried on the paper discs. Each data point was repeated three times to generate standard deviations.

DNA Amplification Procedure

After extraction and quantitation, thermal cycling was performed using a Veriti thermal cycler from Applied Biosystems. The whole final filter disc was taken directly to PCR. Thermal cycling protocol was carried out by loading a total PCR volume of 15µl on the filter disc, containing 10µL of AmpFlSTR PCR Mix and 5µL of AmpFlSTR Identifiler Primer Set. Similarly, positive and negative control tubes were prepared, adding 10µl of 9947A Standard (Amp FL STR Identifiler Plus) DNA and up the final reaction volume to 25µl. The program includes an initial activation of Taq polymerase at 95 ºC for 5 min, followed by 35 cycles at 95 ºC for 30 s, 60 ºC for 30 s, and 72 ºC for 30 s, and a final extension step for 10 min at 72 ºC. All amplified DNA samples were analyzed off-chip using standard gel electrophoresis techniques.

STR Typing and DNA Sequencing of extracted DNA

PCR products were analyzed off-chip by capillary gel electrophoresis on the Applied Bio 3500 Series Genetic Analyzer. A typical STR assay, which involves simultaneous detection of more than 15 STR loci, was performed by adding 25µl formamide: GeneScan™ 500 LIZ® Size Standard mixture into the well-containing PCR product and then loading the plate for electrophoresis run to generate an electropherogram.

Optical detection of On-Chip extracted DNA

To carry out optical detection of amplified PCR product, Fusion-5 filter paper containing quantitated DNA was immersed in an aqueous solution of ethidium bromide staining dye at room temperature for 2 minutes and then excited with a blue or UV LED using a UV Trans-illuminator.

RESULTS AND DISCUSSION

Optimization of the PMMA membrane based on the Concentration of PMMA

To optimize the thickness of PMMA membranes for efficient DNA capture, PMMA membranes of 2%, 3%, 5%, 6%, and 8% by (PMMA+PVP) weight were made. Among all concentrations, based on the results of the characterization studies, a PMMA membrane of 5% by weight (Figure 2) was found suitable for assembly into the Microchip.

SEM Imaging Analysis

Microscopy is the direct method for pore size determination. It can visualize large pores and defects that are not evident otherwise. The surface morphology of PMMA films of different

Figure 2. PMMA membrane (5% by PMMA+PVP wt.)

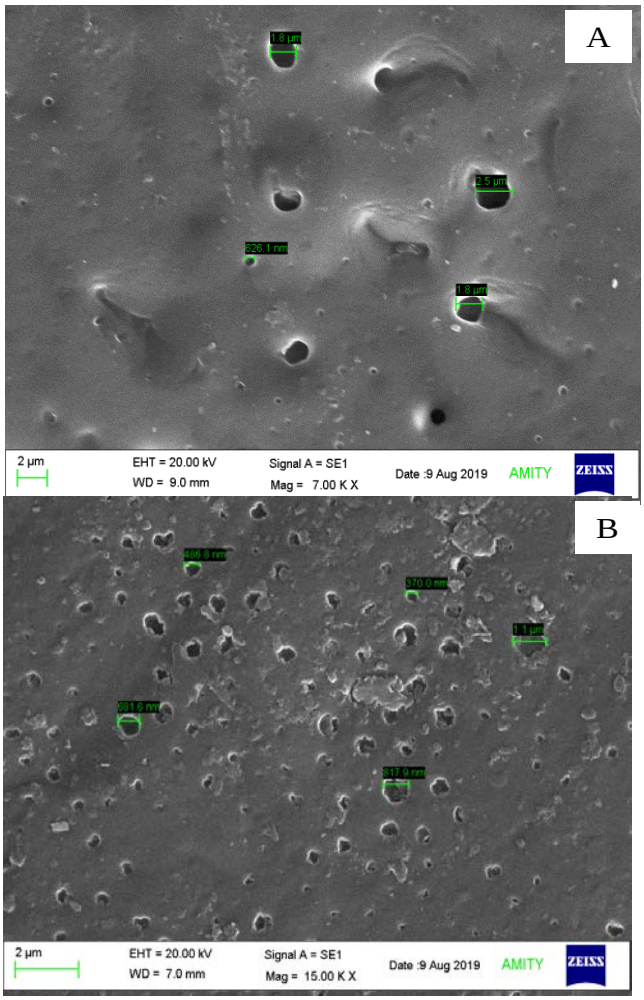


Figure 3. SEM Images showing surface morphology of PMMA membranes **(A)** at 5% PMMA+PVP and **(B)** at 8% PMMA+PVP.

concentrations of PMMA+PVP (2%, 3%, 5%, 6%, and 8%) was analyzed. An SEM image of the PMMA film at a higher concentration indicates that it does not accept the high voltage source because of its thickness, as bubbles are seen in the SEM image at higher concentrations. The PMMA surface morphology shows a rough surface and micro-pore structure (Figure 3A). The surface morphology changes severely, from smooth to rough, as the % age of PMMA+PVP increases and reaches, at best, a specific concentration. The surface morphology at 5% by wt. of PMMA+PVP is a smooth matrix of the blend film, which is evidence of satisfactory miscibility between the two polymer matrices. Smooth morphology closely relates to the interaction between two matrices of PVP and PMMA due to cross-linking, which indicates that the blend is compatible. At a higher (8% by wt.) content of PMMA+PVP, the surface of the PMMA membrane is found to be rough within a homogenous composite matrix. Therefore, the membrane has the best cross-section morphology at a 5% wt. concentration of PMMA+PVP (Figure 3B).

FTIR Results

FTIR Spectroscopy is a vital spectroscopic tool in the investigation of polymer structures. It determines the occurrence of complexation/ interaction between two polymer matrices.[20–22] Infrared spectra reported in this work were taken with a spectrophotometer in the wave number region between 3500 and 500 cm^{-1}. (Figure 4) shows the FTIR spectrum of the PMMA film (a) and the fused PMMA-Fusion-5 filter film (b). The first graph of PMMA film shows a sharp, intense peak at 1722.3 cm¯1, which appeared due to ester carbonyl group stretching vibration. The broad peak ranging from 1230 – 1000 cm¯1 can be explained owing to the C-O (ester bond) stretching vibration. The two bands at 1388 cm¯1 and 749.87 cm¯1 can be attributed to the alpha-methyl group vibrations. The band at 976.9 cm¯1, 1062 cm¯1, and 840 cm¯1 is the characteristic absorption vibration of PMMA. The band at 1439 cm¯1 is attributed to the C-H bonds of the CH3 group bending vibrations. The two bands at 2996.82 cm¯1 and 2948.9 cm¯1 can be assigned to the C-H bond stretching vibration of the CH3 and CH2 groups, respectively.

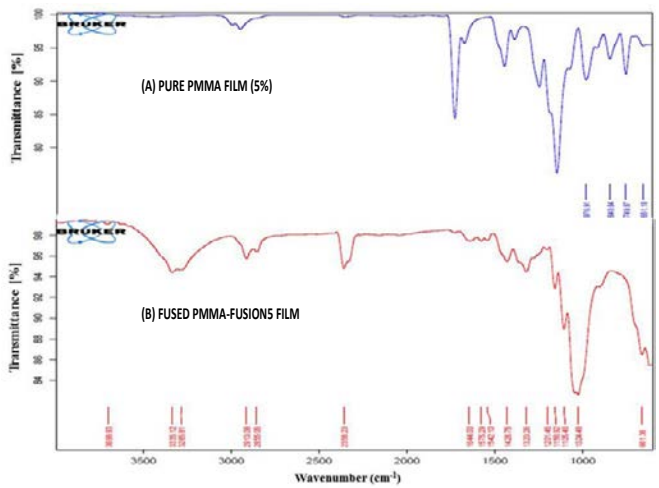


Figure 4. FTIR Spectrum image of (a) 5% Pure PMMA (b) Fused PMMA-FUSION5 Filter Disc;

XRD Study Results

The present study uses the XRD method to study the polymers' amorphous, crystalline, or semi-crystalline nature. The XRD of Pure PMMA (5%) film and fused PMMA+ Fusion-5 filter disc is shown in (Figure 5). The semi-crystalline phase of PMMA is evident at 21 ºC and 24.7 ºC. Adding PMMA+PVP indicates a decrease in the relative intensity of the peaks, stating that the amorphous phase increases in blend films.

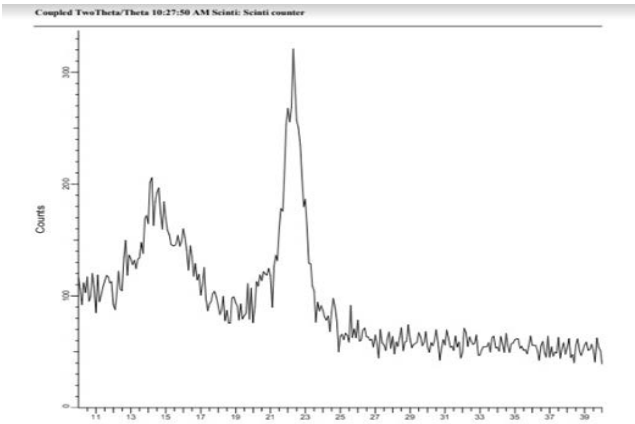
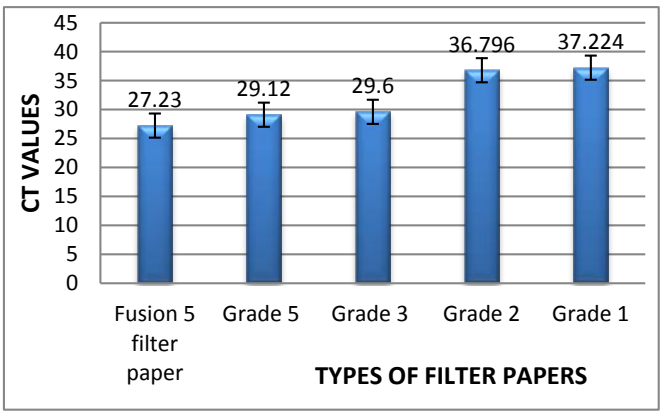


Figure 5. X-RD Pattern of Membrane of 5% Wt. PMMA+PVP

OPTIMIZATION OF THE ON-CHIP DNA EXTRACTION

We first optimized the operation process of DNA purification on the microdevice. Many parameters may affect the performance of on-chip DNA extraction. Among them, the selection of filter paper was identified as the most important one that can be thoroughly optimized.

Table 2. Comparison of CT Values of different types of filter papers



We compared the DNA capture capabilities of different types of filter papers, including Fusion-5 and Whatman filter paper grades (1, 2, 3, and 5), with particle retention sizes ranging from less than 2µm to 11µm. Ten nanograms of 9947A Standard (Amp FL STR Identifiler Plus) DNA with a one ng/µl concentration was pipetted onto the filter discs and washed using TE, followed by real-time PCR quantification. As shown in Table 2, the real-time PCR results demonstrated that the

Fusion-5 filter paper provided the highest DNA capture efficiency. This result illustrated that the average particle retention size of the filter paper is related to the DNA capture efficiency.

The threshold cycle (CT) value for each sample was determined. A CT value of 27.2 or less than that obtained through other filter papers, as shown in Table 2, indicates the high entanglement of DNA molecules leading to high DNA capture efficiency. Thus, we inferred that filter paper with too big pores cannot be entangled with DNA molecules efficiently, while one with too small will trap more PCR inhibitors. In addition, the capture efficiency is also affected by the compositions of the filters, as Fusion-5 is quite different from the other cellulose-based filters. Other reasons that make the Fusion-5 filter the best choice include its hydrophilic properties and fast wicking speed, which makes it the best matrix for DNA.

DNA extraction from human samples and evaluation of Capture efficiency.

It is an efficient means for solubilization of proteins. Alkaline lysis at 65 ºC or 95 ºC has been devised to extract single cells and viral DNA in human tissues. However, alkaline extraction of genomic human DNA has not yet been widely used. Here, efficient DNA extraction from four different types of forensic samples was achieved using alkaline treatment at RT in a time frame of as short as 7 minutes.

The mechanism of DNA extraction from the blood by a piece of filter paper is to be as follows: cells in the blood are first trapped by the filter paper and then lysed by NaOH. Exposed genomic DNA gets trapped in the filter paper because long DNA molecules get entangled with the fibers of the filter paper. Cell debris, hemoglobin, and other PCR inhibitors are all washed through the paper during the neutralizing and washing steps with HCl and DI water. Thus, this on-chip DNA extraction process is fast and automated using the program-controlled pump and valve. In addition, inexpensive extraction reagents and low-cost plastic and paper materials for chip construction make the microdevice genuinely cheap.

Table 3. On-chip DNA extraction yields from four different types of forensic samples using real-time PCR quantitation

S.NO.	SAMPLE	C_T MEAN VALUE	QUANTITY MEAN (ng/µl)
1.	Human Whole Blood	29.564	0.6
2.	Saliva	32.909	0.2
3.	Buccal Swabs	36.103	0.18
4.	Dried Blood Stain	37.224	0.4

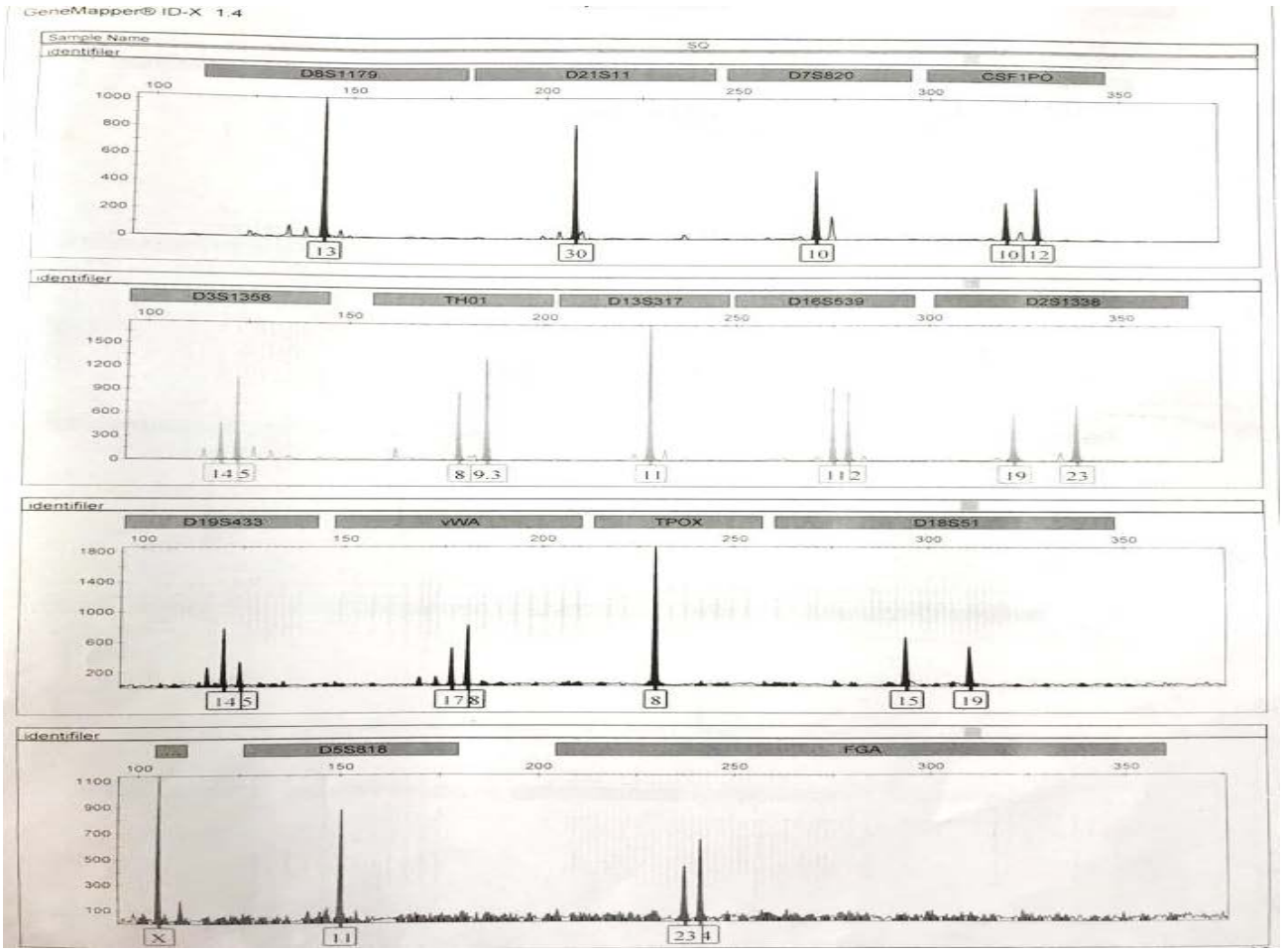


Figure 6. Electropherogram of PCR product of DNA from 2 µl human whole blood on the microdevice

The efficacy of the microfluidic process was evaluated by quantification using Applied Biosystems Quantifiler Duos Kit software. This was performed on Fusion-5 papers containing DNA extracted using this fabricated Microchip from four different types of clinical samples. Quantitation values against the Duo Human target were studied with a calibration curve generated from standard DNA. On-chip DNA Extraction efficiencies from the most encountered forensic samples were evaluated based on concentrations of DNA per µl obtained and CT values, as shown in (Table 3).

After quantitation, the entire chip was taken and put into Eppendorf tubes for PCR amplification. Since the entire DNA extract is trapped in filter paper and can be used as a whole directly for PCR, the template concentration obtained through on-chip DNA extraction is much higher than those 0.1-0.23 ng/µl concentration obtained in the conventional techniques, demonstrating the effectiveness of the on-chip DNA extraction and the severe inhibition effect of whole blood on PCR. Using a modified protocol to bond the fusion-5 paper between PMMA layers, our disposable Microchip withstood the PCR process without any leakage.

STR typing Analysis of Extracted DNA

A typical STR assay was performed after Direct STR amplification of the DNA template on Fusion-5 paper using Amp F1 STR Identifiler Plus PCR amp kit. As shown in (Figure 6), an electropherogram showing a complete and balanced STR profile was obtained from the DNA extracted from a human whole blood sample, proving that 2µl of blood can provide adequate DNA templates using the proposed On-chip DNA extraction technique.

Optical Detection of DNA On-Chip

Visualizing DNA involves labeling DNA molecules with fluorescent stains as they are susceptible and make it easy to visualize small fragments of DNA. Ethidium Bromide (EtBr) is the most commonly fluorescent DNA stain. Individual EtBr molecules can get squeezed between adjacent base pairs of a DNA double helix called intercalation. When excited with UV light, any EtBr intercalated into the DNA fluoresces, producing a bright orange light.

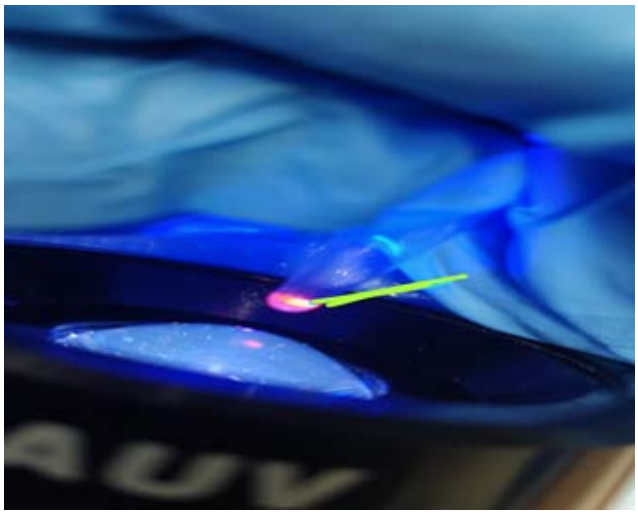


Figure 7. Color fluorescence shown by Captured DNA on Fusion-5 paper under UV light

To visualize DNA trapped on the Fusion-5 filter paper, it was immersed in ethidium bromide solution and then taken under a powered UV torch to excite the fluorescent DNA molecules (Figure 7).

CONCLUSIONS

This study presents the successful integration of the fused film of PMMA and Fusion-5 in the micro-fluidic device developed for On-chip DNA extraction. Direct inclusion of Fusion-5 within the system has provided successful DNA extraction from human whole blood within 7 minutes. Besides that, a direct DNA extraction using a Fusion-5 filter containing various other raw samples like dried blood stains and buccal swabs was also done, resulting in higher DNA yield, demonstrating the effectiveness of the On-Chip DNA extraction. Further, a fast, sensitive, cost-effective DNA detection tool based on Fusion-5 paper that can display immediate color without any instrumentation was developed, making on-site testing possible. The inherent advantage of using Fusion-5 paper within the proposed system is that it reduces the risk of sample contamination, which is ideal for solid-phase nucleic acid isolation, off-chip PCR amplification, and optical detection.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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