An automatic integrated microfluidic device for DNA extraction using electric valves

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Abstract

Deoxyribonucleic Acid (DNA) is a molecule containing genetic instructions that influences the development and function of living organisms. Understanding DNA and its properties is essential for various analytical techniques, such as DNA sequencing, and genetic testing. DNA extraction is a fundamental process that allows scientists to access the genetic information in DNA. There are various methods for DNA extraction from a biological sample which cell lysis and DNA purification are two common steps of them. The chemical cell lysis is selected for this study and a passive micromixer has the microreactor role to mix the lysis buffer and input sample. Also, a silica-based filter is selected for DNA purification. Different units of DNA extraction method are integrated into the fabricated device which includes mixing, lysis and DNA extraction microfluidic chips which are assembled on a chip and form a lab-on-a-chip system that are connected together using electric valves (To open/close system valves to change the flow of different fluids). To optimize the device different parameters including fluid velocity, filter thickness, temperature and cell concentration were studied and the DNA concentration for the optimum values was introduced for 2.5 million cells per milliliter at 65˚C with one silica filter and a vacuum chamber with 25 milliliters. The highest yield for extracted DNA was reported at 147±36 ng/µl, which had a ~90% extraction efficiency in comparison with the reference DNA extraction kit. Finally, the quality of seven samples of extracted DNA from the device was studied using PCR and Gel electrophoresis tests.

Keywords: DNA Extraction, Chemical Cell Lysis, Microfluidics, Electric Valves, Lab-on-a-chip System

1 Introduction

DNA is a molecule that contains the genetic instructions for the development and functioning of all known living organisms and is composed of smaller units called nucleotides[1]. It serves as the hereditary material, carrying the information needed to build and maintain an organism's cells and direct their activities and their structure is often described as a double helix[2]. DNA technology and research have a profound impact on various fields, including genetics and medicine[3]. The study of DNA helps scientists to understand the mechanisms of genetic diseases, develop diagnostic tests, create genetically modified organisms, and even solve

crimes through DNA profiling[4]. In fact, DNA is a remarkable molecule that holds the key to the inheritance and diversity of life on Earth and its structure and function have revolutionized our understanding of biology.

DNA extraction is a fundamental step in various scientific and practical applications that involve the study or manipulation of DNA that is routinely undertaken in biological academic and research settings. The DNA extraction process is done using different methods[5], according to the type of sample the final goal[6]. Choosing the right method for DNA extraction is a very critical step because the quality and quantity of DNA affect the success and reliability of any analysis[7]. In order to extract the DNA from a biological sample, its cells should be lysed; Cell lysis is a process that breaks down the cell membrane to release intracellular materials such as DNA, RNA, and proteins. In fact, this process is a very important and necessary part of the diagnosis of the disease[8]. In this process, the cell membrane is ruptured, and the cell nucleus containing the genetic information is exposed and the DNA molecules are extracted[9]. Different methods can be used to achieve cell lysis, depending on the type of sample and the desired downstream applications each of which has differences from each other. These methods include chemical[10], electrical[11], mechanical[12], laser[13] and thermal[14] methods.

Chemical lysis has received a lot of attention in microfluidic applications due to its lack of complexity and simple microstructure, and it is considered as a suitable commercial source for cell lysis[15]. This method includes the use of chemicals or special solutions to disrupt cell membranes and release cell contents, including DNA[16]. The chemical lysis method is a simple and low-cost method because only a lysis reagent is required, and for this reason, this method is most effective in microfluidics[17]. In different studies, researchers compared the efficiency of lysis buffers and different detergents. Chen et al. compared the percentage and efficiency of guanidine lysis and Triton X-100 lysis buffer using a sandwich type microfluidic device, and their results showed that the time required for complete cell lysis by guanidine salt is about 174 s, by Triton X-100 about 161 s and by Mixture buffer C is about 108 s. They also concluded that by increasing the cell flow rate, the solution flow time decreases and as a result, the cell lysis process worsens[18].

Microfluidic technology offers precise control and manipulation of liquids on a small scale, enabling efficient cell lysis and mixing, and integrating multiple functions and processes into a single platform. The integration of cell lysis and mixing functions enables efficient sample preparation, reduced sample volume, reagent consumption, and also enables high-throughput

automated processes that make them suitable for a variety of applications. By using effective mixing, the whole process of cell lysis, DNA extraction, purification and washing can be facilitated[19]. For this purpose, various types of microfluidic mixing techniques have been studied in the last few decades[20]. The two main types of mixing include active and passive mixing[21]. In active mixing, external energy sources are used to disrupt fluids[22], which include forces such as acoustic[23], magnetic[24], dielectrophoretic[25] and thermal[26] forces. Another type of mixing process is passive mixing, which is also used for cell lysis[27]. Zhu et al. developed an inertial microfluidic system for WBC extraction from whole blood. In this system, the process of lysing cells is done by the mixer part, which mixes the cell solution with the lysis reagent[28]. In addition, Bahrami et al introduced a spiral micromixer in order to increase mixing efficiency, whose channel walls are sinusoidal. The results of their research showed that with the design of this micromixer, the mixing index increases by 99.11%[29].

DNA extraction common methods include various activities in a laboratory by macroscale facilities such as centrifuge, heart, shaker or incubator. On the other hand, the lab-on-a-chip DNA extraction method is one of the advantages of microfluidic technology to perform efficient and automatic DNA extraction from different types of samples that integrate several DNA extraction steps such as lysis, binding and washing in a single microfluidic device. This method uses laboratory protocols that are usually used by biologists, and its processes usually include sample filtration, centrifugation, distillation, dilution, and sample extraction[30]. The use of this method includes advantages such as reducing sample and reagent volume, faster processing times, precise control of fluid flow and mixing, and can be performed automatically and with high throughput[31]. Chen et al. designed a device that can perform various processes including cell separation, cell lysis, and DNA purification during a continuous flow process. They compared the percentage of lysis and their efficiency using a microfluidic device for the lysis of mouse blood cells. Their results showed that the higher the cell flow speed, the shorter the solution flow time, and as a result, it causes unfavorable cell lysis[18]. In another study, Karle et al. designed a novel microfluidic platform using phase-transfer magnetophoresis that enables continuous biomolecule processing that demonstrates continuous DNA extraction from Cell lysis on a microfluidic chip and can be a complement to Continuous flow PCR. Their results showed that this platform has fast turnaround time for DNA extraction and high DNA recovery compared to traditional batch extraction methods[32].

In this study, all the required processes for DNA extraction are integrated in a device, which automatically perform the steps. The device consists of three microfluidic chips including

mixing, lysis and extraction chips, which are related by electric valves. A controller switches the valves in each step to guide the fluids to pass through a filter in the extraction chip, respectively. To optimize the DNA concentration, different values for number of filters, fluid velocity, temperature and cell concentration are analyzed. Additionally, PCR and Gel electrophoresis test is performed to check the quality of extracted DNA.

2 Device Principle and Theory

In this study, a commercial DNA extraction kit is utilized to extract DNA from cells. In this kit, firstly, 200 µl of cell suspension is mixed with 200 µl of lysis buffer and 20 µl proteinase K. In order to lysis the cells, this mixture is maintained at 65˚C for ten minutes, then, 200 µl of pure ethanol is added to it. In the next step, the mixture should be placed in a silica filter column, and be centrifuged to pass through the filter which has affinity with intracellular materials. Next, the filter is moved to another column and 1st washing buffer should be passed through the filter using a centrifuge to wash undesirable materials. This step, then, is repeated using 2nd washing buffer to eliminate all the components except DNA. Finally, the elusion buffer washes all the DNA attached to the silica filter and stored at the end of a microtube. Figure 1 illustrates the summary of this protocol using the usual facilities in a laboratory.

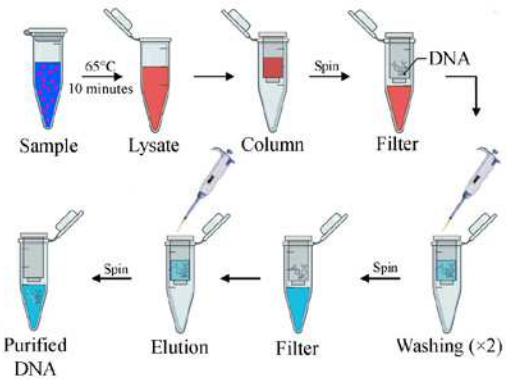

Figure 1. DNA extraction protocol from the cell samples to purified DNA. The sample is heated after ten minutes and turned to lysate which is placed in a silica column. Then it is centrifuged to pass through the filter. This centrifugal step is repeated with two washing buffers and the elution buffer.

As shown in Figure 2, the designed device is a system including electric valves, microfluidic chips and a thermal module. In the first, sample cells and lysis buffer pump in the mixing chip

using a syringe pump out of the system which is connected to the green tubes in the figure, and the mixed fluids are sent to the next chip using the tube. The Second chip is named as lysis chip because the lysate is created in the reaction chamber. In order to prepare the suitable temperature for the lysis process, an electric heater is integrated under the two first chambers; the second chamber is in the same heating condition with the reaction chamber to control the temperature, since a thermometer sends feedback to a controller which turns of the heater if the temperature reach 65˚C. After ten minutes, pure ethanol is added to the reaction chamber, and other buffers including the first and second washing buffers and the elution buffer are loaded into the buffer chambers. In the next step, the other syringe, out of the device, produces a vacuum chamber which is connected to the red tube named waste vacuum outlet in Figure 2. At this moment the number 1 and 6 valves are opened, and then the lysate passes through the silica filter integrated in the extraction chip and guided to the waste vacuum outlet. Then, the 6^{th} valve is switched; the waste fluid is pumped into the system and goes to the waste microtube. Afterward, the 6^{th} valve is switched back, and the waste vacuum outlet sucks the first washing buffer through the 2^{nd} valve which is open now. This step is repeated for the second washing buffer too, and the two washing buffers eliminate the other materials except DNA from the silica filter domain. Following this, the syringe connected to the target vacuum outlet sucks the elution buffer passing through the 4^{th} valve, silica filter, and 7^{th} valve and washes all the pure DNA which were stored in the silica filter. Subsequently, the 7^{th} valve is switched, and purified DNA is sent to the target microtube pumping from the syringe connected to the target vacuum outlet. Later, the ethanol stored in the last chamber of the lysis chip is circulated inside the device to wash the valves, and finally, the chips are exchanged for the next test.

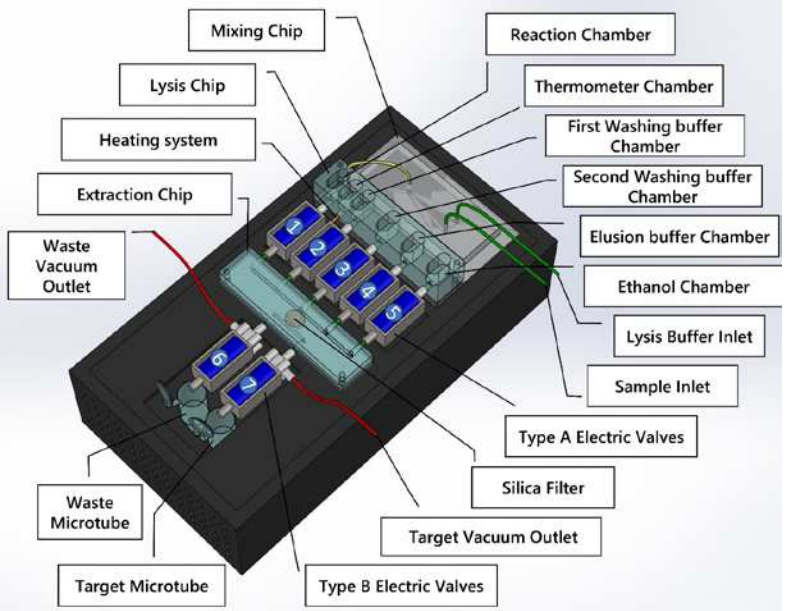

Figure 2. Schematic of the designed device including different microfluidic chips, valving and thermal system for flow fluid control.

3 Methods

3.1 Materials preparation

The input sample for DNA extraction is MCF-7 breast cancer cells which were obtained from Stem Cell Technology Research Center, Tehran, Iran. The cells were grown in 10 cm culture dishes and were maintained in DMEM with 10% heat-inactivated fetal bovine serum. DynaBio™ Blood/Tissue DNA Extraction Mini Kit was purchased from Takapouzist company, Tehran, Iran; this kit includes all the buffers used in the protocol.

The device body, lysis and extraction chips are made from Polymethyl methacrylate (PMMA) material which is prepared from a local market. Electric valves were purchased from Module-center shop, Tehran, Iran. Moreover, other electric modules such as controller, relay, thermometer and heater are prepared from Jahan Kit shop, Tehran, Iran.

3.2 Spectrophotometric analyses of DNA

The concentration and purity of extracted DNA are important parameters in DNA extraction for downstream analyses to be accurate and reliable. Analyzing the absorbance ratio at 260/280 nm and 260/230 nm is a common method for determining DNA purity. As a result of the 260/280 ratio, it determines how much DNA and protein contamination is present in the sample, with a ratio of ~1.8 indicating pure DNA. The 260/230 ratio reflects the presence of

contaminants such as salts and organic compounds, with a ratio of ~2.0 indicating minimal contamination. In this study, the concentration and purity (260/280 and 260/230 ratios) of the extracted DNA were measured with a NanoDrop™ Take3 plate of Cytation™3 (Bio Tek, Agilent Technologies, Inc, USA), using 2 µL of each sample.

3.3 Agarose gel electrophoresis

The DNA integrity was analyzed by agarose gel electrophoresis using 1% agarose for genomic DNA (gDNA). Ethidium bromide (EtBr) was used to stain the agarose gel. Electrophoresis was performed utilizing 1x TAE (Tris Acetate-EDTA) buffer and a constant voltage of 100 V for 40 min. We visualized DNA bands and acquired images using a Gel Doc (UVITEC Cambridge).

3.4 PCR amplification

For PCR analysis, each DNA sample was diluted to a working concentration, and performed in the device Steponeplus™, Applied Biosystems, USA. The amplification was performed by a housekeeping gene with the forward primer GAPDH: 5′- TTCAACAGCGACACCCACTC-3′ and reverse primer GAPDH: 5′- GAAGATGAAAAGAGTTGTCAGGGC-3. PCR reactions were carried out in a final volume of 15 µL that contained the following: Master mix 2x 7.5 µL (Master mix 2x SMOBIO™, Technology, Inc, Denmark), 1 µL of DNA template (0.1–10 ng/µL),0.5 µL of each forward and reverse primers (5 µM), and 5.5 µL of sterile water. PCR thermal cycling conditions were as follows: pre-denaturation at 95 °C for 10 min, followed by 40 cycles of denaturing at 95 °C for 30 s, annealing at 60 °C for 30 s for GAPDH, extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min.

3.5 Microfluidic Chip Fabrication

There are three different chips including mixing chip, lysis chip and extraction chip. The mixing chip is fabricated by polydimethylsiloxane (PDMS) material and standard photolithography[33] because the designed channel had sharp and small features which is shown in Figure 3a. On the other hand, as the lysis and extraction chips did not have small features which are shown in Figure 3b and Figure 3c, PMMA is utilized to fabricate them by CNC machine (Roland MDX 40A, Japan). Figure 3d depicts the lysis chip layers, which will be bonded using chloroform and aligner holes. The lower layer contains horizontal holes as an outlet for this chip toward electric valves. Figure 3e illustrates different layers of the extraction chip. The upper layer includes five horizontal holes as inlets of this chip, which are connected by a channel. The circle in the middle layer is considered for the silica filter which all the input

fluids should pass through that. The lower layer of this chip includes two connected outlets that guide output materials to two different valves.

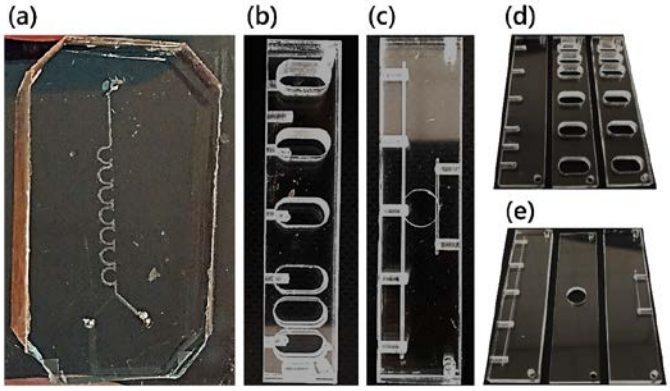

Figure 3. Microfluidic chips utilized in the device. a) Mixing chip containing omega-shaped arrays as the micromixer, b) lysis chip with different chambers for buffers, c) extraction chip including a circle chamber for silica filter, d) lysis chip and e) extraction chip layers before the assembly.

3.6 Chip-valve connectors

In order to connect electric valves, which are permanent units of the system, to the disposable chips, a connector was needed to eliminate leakage possibility; since the fluid moves thanks to vacuum pressure, a special 3D printed part was designed and fabricated that is shown in Figure 4. The connectors were designed with a secure fit within the channels, effectively preventing any potential leakage. To achieve this, the connectors feature multiple narrow protrusions along their axis on both sides. Notably, the thickness of these protrusions decreases from the end to the top, maximizing the contact area under pressure. For the fabrication of connectors, Anycubic Tough flexible epoxy and the Anycubic Photon M3 Plus 3D printer were employed. The optimal number of protrusions and the flexibility of the epoxy ensure optimal contact between the connectors and the inlets/outlets, resulting in a 0% leakage rate. Figure 4a and Figure 4b show the 3D and 2D design of connectors which indicate the ability of connectors to eliminate leakage because not only their material is flexible, but also the protrusions are designed to bend more when going to the holes of valves and chips. As shown in Figure 4c two types of connectors were employed in this study: one for linking the valves to tubes and another for connecting the valves to the chip, as well as for interconnecting different chips.

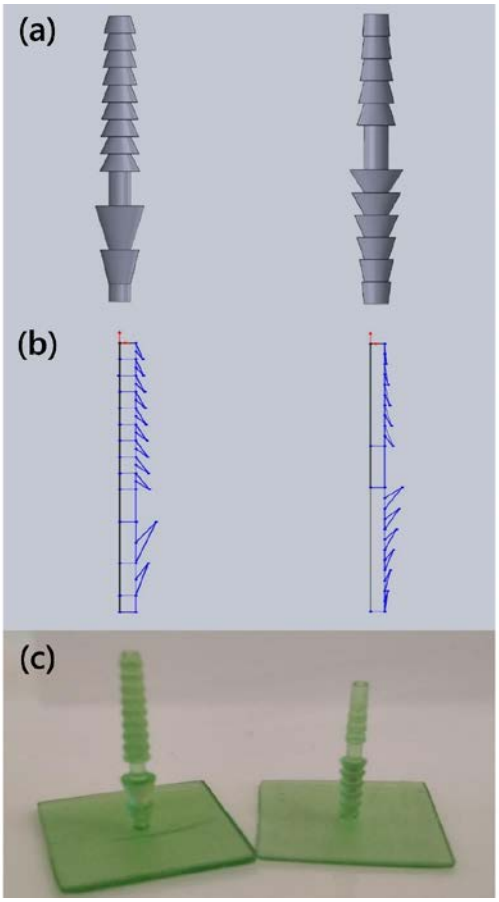

Figure 4. a) 3D design and b) 2D sketch of connectors, and c) fabricated connectors using 3D printing method

3.7 Control system

Heat transfer and fluid flow in the system should be controlled. A constant temperature of 65˚C for ten minutes is needed during the lysis process. Therefore, an electric module integrated under the lysis chip generates heat, and simultaneously, a thermometer inside the reservoir sends feedback to the controller (Arduino Uno). Then, the controller turns off/on the heater based on the feedback using a relay, and maintains the temperature at a constant value.

The fluid movement has two steps; first, the syringe pump (Fanavaran Nano-Meghyas, Tehran, Iran) pumps the samples into the lysis chip. Secondly, another syringe pump sucks the fluids in five steps; each electric valve is opened by the controller in its desired step. Therefore, fluid flow is controlled by Arduino and moves inside the channels by syringe pumps. It should be noted that all the steps are switched to the next one using a button integrated in the control system.

4 Result and discussion

4.1 Mixing

The first unit of the integrated system is the mixing chip that is shown in Figure 5a, and utilized to blend the cell sample and lysis buffers. The designed geometry consists of curved and sharp edges which are the reason for secondary flow generation[34]; this kind of flow increases mixing quality because its direction is orthogonal to the main flow direction[35]. Therefore, the two fluids passing through the channel will be mixed better than a channel without secondary flows[36]. Figure 5b-f illustrates the mixing test using food color to analyze the quality of the designed geometry. As illustrated in Figure 5b, two fluids are completely separated entering the channel; they will be mixed while they are going toward the outlet. It is qualitatively shown that the complete mixing happened at the outlet (Figure 5c). However, although this qualified result is acceptable for the application, we also extract the quantified data from the figures using a simple image processing method. In this method, the inlet picture is considered as the reference which the blue color is quantified to the number 100 and the water color is quantified to 0; it means the complete mixing should show the middle number of 50. Then, all the pixels from the outlet picture are checked to find their standard deviation (σ) from 50. The standard deviation for different repetitions was lower than two, which means approximately all the pixels show a complete mixing.

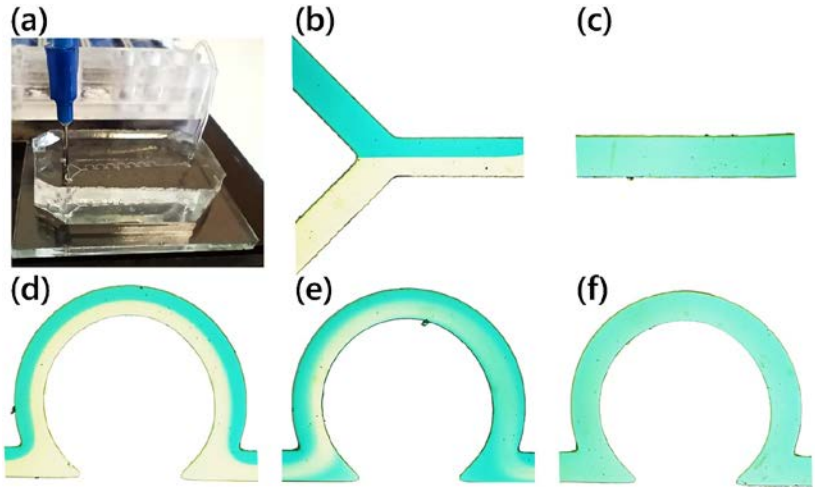

Figure 5. The mixing system. a) The connections and location of the mixing chip in the integrated device; the outlet is connected to the lysis chip using a tube. b) Inlet condition and c) outlet condition for the mixing chip. d) Mixing condition for second, e) fourth, and f) sixth Omega unit.

4.2 Heating system

After the mixing step, the mixture of cells and lysis buffers stays in the lysis chamber for ten minutes. As mentioned before, the heating system should be controlled to maintain the

temperature at 65˚C. Figure 6 illustrates the result of the control system; after the electric heater is turned on, the temperature rises up to the thermometer show 65 ˚C, and then, the heater is turned off but the temperature experiences an overshoot because the heater power is high. Afterwards, the temperature falls and when it reaches 65˚C, the heater is turned on again. In this heating system, a relay turns on and turns off the heater alternatively and keeps the temperature constant. According to the result, after two minutes the temperature gets constant at the desired value which is required for lysis reaction.

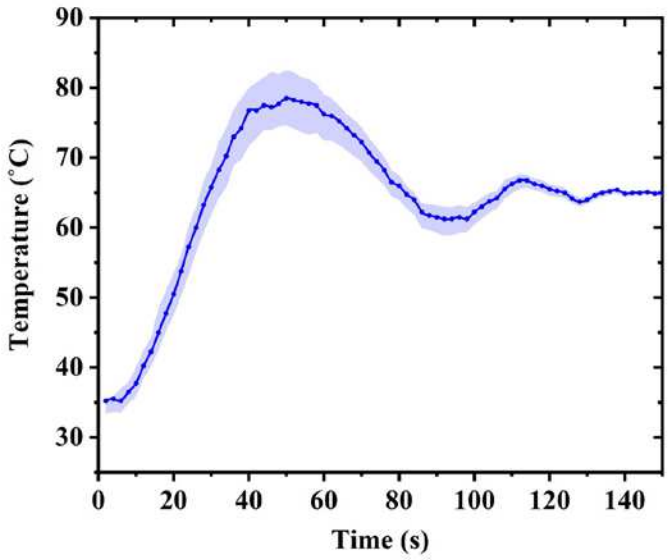

Figure 6. The lysis chamber temperature after starting heating the chamber. The temperature is controlled by turning off/on the heater using a relay.

4.3 Electric valves

There are two syringes connected to the outlets which create two vacuum chambers including target and waste chambers. Figure 7a indicates these vacuum chambers that suck the fluids which are loaded before in the lysis chip. Figure 7 illustrates the process using some food colors in order to elaborate each step; in Figure 7b, the first state of the lysis and extraction chips is shown. Firstly, all the materials including lysate, washing buffers, elution buffer and ethanol are loaded in the lysis chip. Secondly, the first valve is opened and the vacuum chamber sucks the lysate into the extraction chip and passes it from the silica filter which is shown in Figure 7c. In this step, the DNA and other impurities attach to the filter. Thirdly, just the second valve is opened and the first washing buffer washed a part of the impurities and guides them to the waste chamber; this step is shown in Figure 7d. Based on Figure 7e, the next step begins when the third valve is opened and the second washing buffer is guided to the waste outlet. At the

end of this step, all the impurities are cleared from the silica filter and the purified DNA is stored at the filter domain. Afterward, as illustrated in Figure 7f, the fourth valve is opened and the target vacuum chamber sucks the elution buffer, and collects the purified DNA in the outlet. Finally, the fifth valve is opened, and ethanol enters the network. The purpose of this step is cleaning the valve, therefore, the syringe pump and valves controlled in this step to pass through all valves

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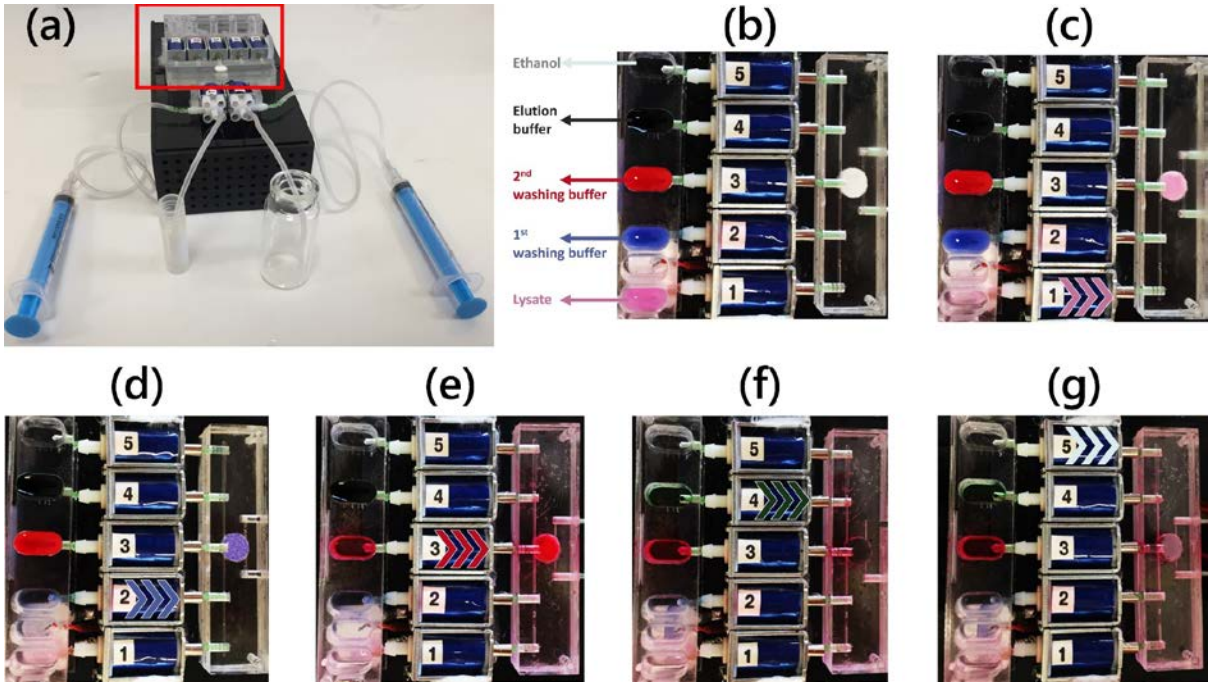

Figure 7. Flow control system by electric valves. a) vacuum syringes connected to the outlets that prepare the required pressure difference to move the fluids in the channels. b) Different colors are loaded to depict the steps of the process. c) The opening of the first valve to release the lysate. d) The opening of the second valve to release the first washing buffer. e) The opening of the third valve to release the second washing buffer. f) The opening of the fourth valve to release the elution buffer. g) The opening of the fifth valve to release the ethanol for washing the system.

4.4 DNA concentration

To optimize DNA extraction in the device, four parameters including vacuum volume, number of silica filters, lysis temperature, and cell concentration are investigated, and the concentration of purified DNA was measured using a Nanodrop device.

4.4.1 Vacuum capacity

The vacuum generator is a syringe in the fabricated device. On the other hand, the size of the utilized syringe plays an important role in fluid suction. If the vacuumed volume is bigger, the suction is stronger, therefore, the fluids pass through the filter easier. As shown in Figure 8, five different volumes are tested for the process. It can be concluded that higher values of

syringe volume are efficient for DNA extraction. The suction strength is directly related to the quality of passing the fluids through the silica filter. In the lower vacuum volumes, there is still a portion of the fluid in the inlet reservoir or in the channels before the filter; it is the reason why the five-milliliter syringe has the lowest concentration. Therefore, if there is no remaining fluid behind the filter, the DNA concentration can be increased. According to the results, this has happened for the volumes higher than five milliliters. There is a slight rise in these volumes that is related to the speed of flow passing through the filter because the higher velocities wash the silica filter better.

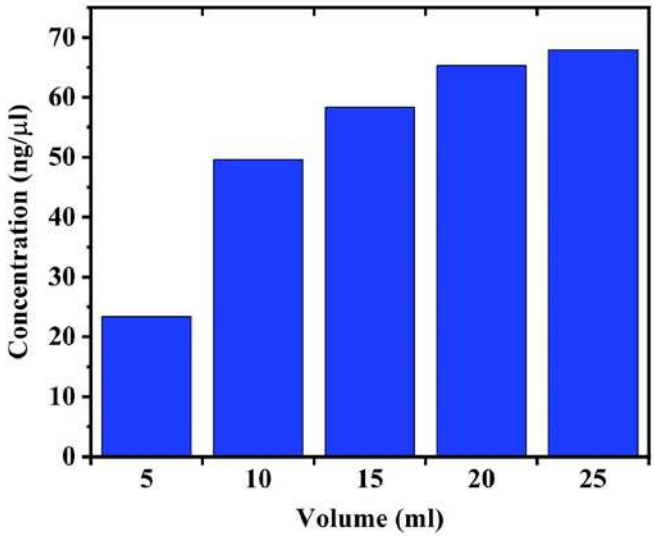

Figure 8. DNA concentration based on the volume of the outlet syringe which creates a vacuum for moving the fluids

4.4.2 Number of filters

The silica filters that are utilized in the extraction chip are the same filter in the extraction columns of the commercial kit. In order to optimize the extraction chip, five different states of filter are integrated in the chip and the outlet concentration is measured which is depicted in Figure 9. Five different thicknesses of silica filter are tested in this study; as the original filter is a circle with a diameter of 7 mm and thickness of 1 mm, the value of 1 mm for the filter thickness is considered as one filter, and other values indicate the number of filters based on their thickness. According to Figure 9, a standard size of the filter results in the maximum concentration for the extracted DNA. The lower thicknesses have two weaknesses; firstly, they do not provide enough space to attract DNA, and secondly, they are mechanically unstable and may be destroyed by fluid pressure; therefore, a part of DNA is guided to the waste chamber. On the other hand, while the number of filters is doubled or tripled, the concentration falls

again. During the experiments, it was observed that all the fluids did not completely pass from the multiplied filters. This is due to the large pressure drop that compact filters create for the fluid flow. As a result, a portion of samples in each step remains before the filter and mixes with the next fluid, and reduces the final purity of the DNA.

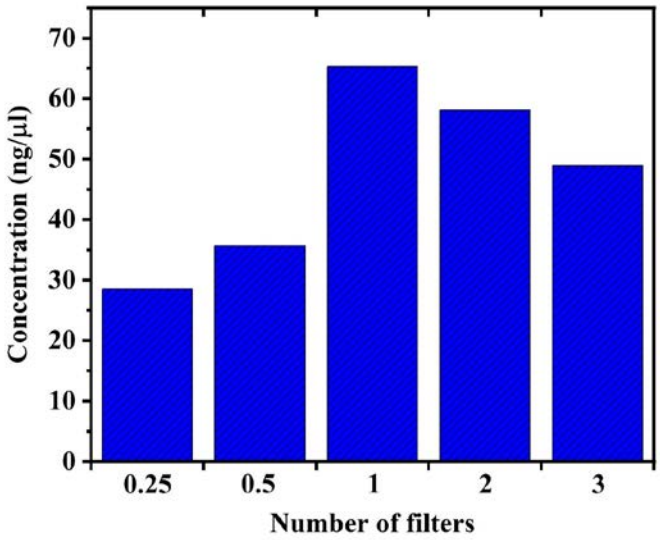

Figure 9. DNA concentration based on the number of filters used in the extraction chip

4.4.3 Temperature and Cell Concentration

Temperature and cell concentration are two other effective factors for the final DNA concentration, which are depicted in Figure 10a and Figure 10b. Heating the sample is a way of improving the lysis reaction, therefore, higher temperatures indicate better concentration. However, the sample should stay ten minutes in an open reservoir on the heater; this is a reason why the higher temperatures cannot be applicable because of sample evaporation. Figure 10a compares the purified DNA with and without heating and a decreased temperature to show that heating can improve DNA concentration which is directly related to lysis step efficiency.

The number of cells or cell concentration has a direct relation with the final DNA concentration. Therefore, different concentrations are utilized to find the optimum concentration. As shown in Figure 10b, the optimum concentration belongs to the concentration of 2.5 million cells per milliliter. It shows the volume of buffers used in the reference extraction kit is optimized for this number of cells, and numbers more than it shows lower DNA concentration. In the column method, increasing the cell concentration over the optimal limit will not result in improved extraction results. Column-based DNA extraction separates nucleic acids based on their physical and chemical properties using solid and stationary phases. Various materials can be used as stationary phases, including silica gel, cellulose, and ion-exchange resins. Silica

column-based DNA extraction methods are saturated because the silica membrane has a limited DNA binding capacity. Therefore, cell concentration does not necessarily increase the amount of DNA extracted

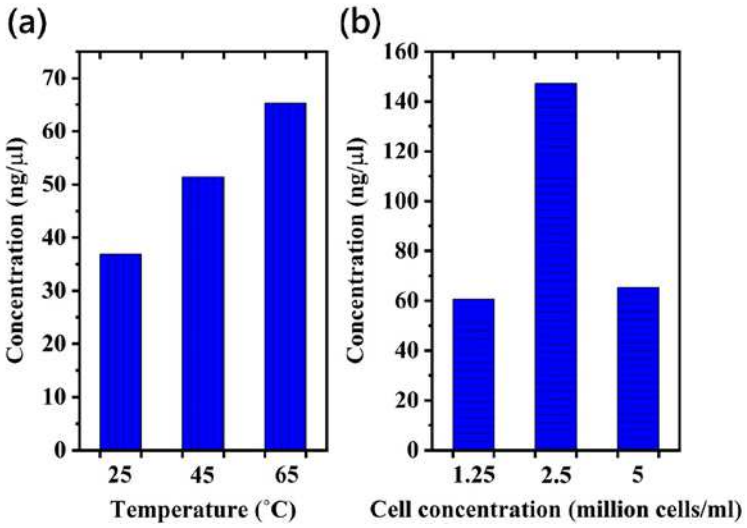

Figure 10. DNA concentration based on factors related to the lysis process including a) lysis temperature, and b) cell concentration

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4.5 DNA quantity and quality

Genomic DNA from cells was extracted using the automated system and the amount of DNA was measured with a spectrophotometer. From a sample of 2.5 million cells per milliliter, 147±36 ng/µl of gDNA was extracted as measured by the Nanodrop instrument with an A260/A280 ratio of 1.534±0.1. These results correspond with a ~90% extraction efficiency with high purity. Gel electrophoresis for the extracted samples revealed a single, high molecular weight DNA band which is illustrated in Figure 11. The input samples were the extracted DNA with different parameters that were investigated in the previous part, and none of the DNA samples showed significant smearing, which indicates degradation of the sample.

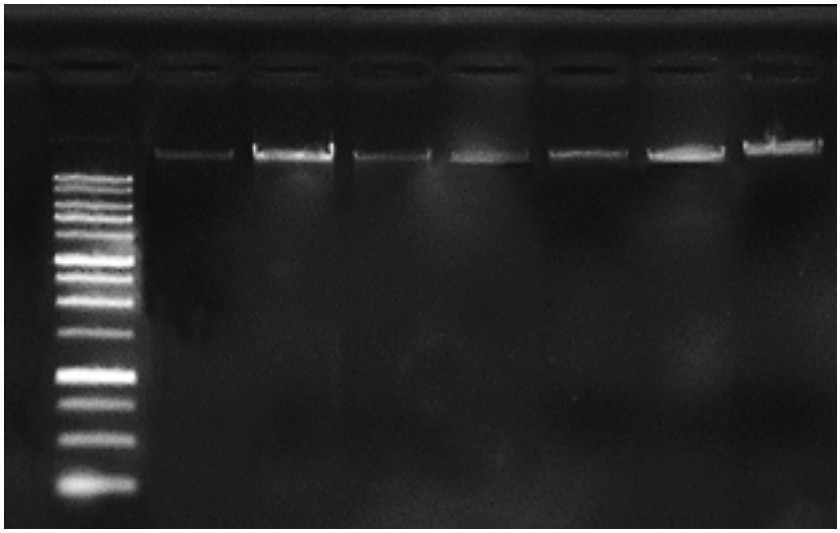

Figure 11. Agarose gel electrophoresis (1%) of genomic DNA. In the left side of the gel is ladder 50bp.

To validate the extraction process further, the extracted DNA was amplified by PCR and compared to a No Template Control (NTC) sample which is a sample without DNA. As illustrated in Figure 12, amplification plots for different extracted samples are displayed. Sample 1 to sample 7 are the extracted DNA using the device with different conditions in their filter, temperature and vacuum condition; All the samples start the amplification between cycle 15 and cycle 20 and finally get stable after cycle 40.

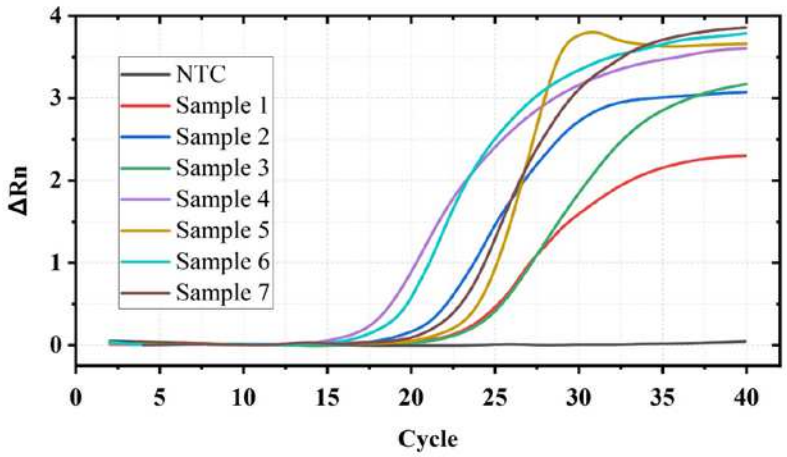

Figure 12. PCR validation. Amplification plots for GAPDH (fluorescence vs. cycles) and comparison of samples with NTC

5 Conclusion

In this study, a novel DNA extraction device was designed and fabricated that consisted of three microfluidic chips including mixing, lysis and extraction chips which were coupled by electric valves. To eliminate the leakage between valves and chips, a 3D-printed part was designed and fabricated with special protrusions to fill the empty spaces. The fluid used in the device moved using external syringe pumps, and the values were switched by a controller to guide the fluids in the right path in the desired step. Different factors in the device were studied to optimize the DNA concentration, and as a result, experiments showed that one silica filter in the extraction chip yields higher DNA concentration. It also resulted that if the fluid passed faster through the filter, DNA concentration increased; it was shown by 25 ml of syringe volume, which produced a stronger vacuum to move the fluid. Although cell concentration and lysis temperature affect the lysis process, their optimum values are introduced as 2.5 million cells per milliliter and 65˚C, respectively. Finally, the optimum DNA concentration was reported 147±36 ng/µl with an A260/A280 ratio of 1.534±0.1 which had an efficiency of ~90 percent in comparison to the standard column DNA extraction method. Moreover, to check the quality of extracted DNA, PCR and Gel electrophoresis tests showed the appropriate quality for them.

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