EXTRACTING SHORT-FRAGMENTED DNA FROM HUMAN BLOOD SERUM SAMPLES USING A NOVEL MICROFLUIDIC CHIP DEVICE

By
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Sc.B., Brown University, Providence, 2015

Thesis

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This thesis by Ga Yeon Lee is accepted in its present form by the Center for Biomedical Engineering, the School of Engineering, and the Division of Biology and Medicine at Brown University as satisfying the thesis requirements for the degree of Master of Science

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Abstract of *Extracting short-fragmented DNA from human blood serum samples using a novel microfluidic chip device*, by Ga Yeon Lee, ScM., Brown University, April 2017

This project aimed (1) to learn the fundamental mechanism of DNA extraction on a novel microfluidic chip that takes advantage of hydrophilic/hydrophobic interactions between sample solutions and channel liquids and (2) to evaluate the feasibility of applying microfluidic chip technology to non-invasive prenatal diagnostics. A simple and efficient method of extracting cell-free fetal DNA circulating in maternal blood serum is necessary to expand applications of non-invasive prenatal diagnostics in clinical settings; a microfluidic chip is a suitable device for point-of-care DNA extraction. In this project, 200 base pair (bp) DNA was used to substitute for cell-free fetal DNA that is characterized by its short, fragmented nature. Three on-chip experiments were conducted: (1) extraction of DNA diluted in TE buffer (10 mM Tris-HCL, 0.1 mN EDTA), (2) extraction of DNA diluted in TE buffer with the addition of carrier beads, and (3) extraction of DNA diluted in human blood serum. With successful recovery of 200 bp DNA at 12.5 – 57.6 % yield in all three experiments, we can ascertain that our microfluidic chip system is suitable for extraction of short-fragmented DNA in TE and human blood serum. When accompanied by advances in microfluidic chip fabrication and DNA extraction, our system could serve as a method of efficient and accurate point-of-care non-invasive prenatal diagnostic.

Background & Introduction

1. Prenatal Diagnostics

Prenatal diagnostics implements various technologies to detect and screen for abnormal genetic conditions of a fetus. In fact, detecting chromosome abnormalities is the main emphasis of most prenatal diagnostics. [1] Gold standard for prenatal diagnostic modalities include amniocentesis and chorionic villous sampling (CVS) which require insertion of needles or devices near the fetus. [2] Although those invasive methods are used extensively, they are considered expensive and are related to loss of fetus during the diagnostic procedure. [3], [4] Miscarriage rate associated with amniocentesis and CVS is around 1—2 % of cases. [5] In an attempt to reduce unfortunate procedure-related miscarriages, many researchers are endeavoring to develop non-invasive and more accurate methods of prenatal diagnostics as a replacement for the aforementioned invasive modalities. [6]

In 1997, the presence of Y chromosome specific sequences in plasma and serum of women pregnant with male fetuses was confirmed, which manifests the existence of cell-free fetal DNA (cff DNA) in maternal serum and plasma. [7] Since the discovery of cff DNA in maternal serum and plasma, much interest in potential application of cff DNA as a non-invasive (or minimally invasive) method of prenatal diagnosis has been generated. [8] Clinical applications of cff DNA include detection of aneuploidies, diagnosis of monogenic diseases, and fetal sex determination. [9] Currently, several methods of quantification of cff DNA has been developed. One quantification method screens for presence of genetic sequences that can only be found in Y chromosomes in male-bearing pregnancies. [9] Another method of cff DNA detection and

quantification selects for fetal single nucleotide polymorphism (SNP) alleles that are passed down from the paternal side. [9] This quantification method is not limited to the gender of the fetus as it can be used for both male or female-bearing pregnancies. Cff DNA is mostly released from the placenta, and they can be detected in maternal plasma after 5—6 weeks of gestation. [10][11] In 2011, the first commercially available cff DNA extraction kit for fetal aneuploidy was implemented for clinical usage. [12] Multiple professional societies, such as the American College of Obstetricians and Gynecologists and the Society for Maternal—Fetal Medicine, have endorsed and strongly recommended usage of cff DNA as a biomarker for prenatal screening and diagnosis for its minimally invasive procedure and low cost. [8], [12] However, there are some challenges to be overcome in order to effectively and efficiently isolate cff DNA from maternal blood serum and plasma. Scientists have discovered that cff DNA makes up only about 3—13 % of the cell-free maternal DNA after 10 weeks of gestation. [12] An original quantitative study reported that 6.2% of the maternal cell-free DNA is comprised of cff DNA. [13] Low cff DNA concentration in maternal serum calls for optimization of cff DNA extraction methods. Moreover, cff DNA on average is shorter than 200 base-pairs which is highly fragmented compared to the size distribution of maternal DNA. [14], [15] Several companies have released cell free DNA extraction kits that are selective for highly fragmented cell free DNA present in low concentration. [16] Although commercially available extraction kits are convenient to use as the reagents are already prepared, they are time-consuming, labor-intensive, and not suitable for point-of-care applications.[17] Electrophoresis has been suggested as a solution to resolve the issue of low-sensitivity, however, separation precision has shown to be low.[18][19] Therefore, development of a cff DNA extraction method that is size-selective as well as suitable for point-of-care applications is crucial to expedite clinical adaptation of cff DNA for non-invasive prenatal diagnosis. In this project, preliminary cff

DNA extraction experiments were conducted on a microfluidic chip device to investigate feasibility of developing a point-of-care cff DNA extraction system that could be further modified to conduct size-selective extraction studies. In this project, 200 bp DNA substituted for cff DNA which is characterized by its short and fragmented length.

2. DNA Extraction

As mentioned above, only 6.2 % of maternal cell-free DNA consists of cff DNA. [13] Therefore, development of efficient and effective cff DNA extraction method is crucial for downstream analysis and universal acceptance of the technology in clinical settings. [20] Multiple studies have compared efficiencies of different cell free DNA extraction kits that are commercially available. [16], [20] Moreover, several labs have published their own extraction protocols over the past few years. [21]–[24] However, there are many limitations with above mentioned protocols – such as multiple washing steps and centrifugation – which make them time consuming and labor-intensive. [17]

One of the most widely used cell free DNA extraction kit is QIAamp® Circulating Nucleic Acid Kit. (Qiagen, Hilden, Germany) [20] This spin column-based nucleic acid purification technology allows cell free DNA to be adsorbed onto the silica membrane (solid phase) while undesired solutes pass through the membrane.[25] [26] It can process about 1—5 ml of sample solution, and elution volume is about 20—150 μl. However, the manufacturer's protocol states that it includes 3 wash steps as well as requires 30 minutes for lysis buffer incubation time. The entire sample processing takes about 2 hours for 24 sample preps. [26] Fully automated high-

throughput version of QIAamp® is available with QIAcube®. [26] However, the system is not scalable nor suitable for low-resource settings due to its expensive cost. [17]

Another way of extracting DNA involves utilization of surface-functionalized magnetic beads. [27] Magnetic beads are suitable for micro-tube format or microfluidic chip DNA extraction for its easiness of separation in solution. [28] Similar to spin column purification principle, magnetic beads are most commonly coated with silica or carboxyl functional groups especially for on-chip DNA separation. [29] When sample solutions containing nucleic acids are mixed with surface functionalized magnetic beads, nucleic acids will bind to the surface of the beads which allows scientists to be able to extract the bead bound nucleic acids using an external magnet. [30] Several silica-based magnetic bead DNA extraction kits are available in the market as they are practical and do not require repeated centrifugation steps, vacuum filtration, or column separation. [28], [31] Additionally, due to electrostatic interactions between silica and chaotropic salts, silica-based magnetic beads can bind to DNA in a non-selective fashion when chaotropic salts are present in the solution. [30] Chaotropic salts facilitate DNA binding with silica surface functionalized magnetic beads by destabilizing DNA molecule's hydrophobic effect. [32]

In this experiment, a novel microfluidic chip designed by Tripathi lab and silica-based magnetic bead DNA separation technology were combined to assess feasibility of extracting short fragmented DNA (diluted in TE and human blood serum) on the microfluidic chip device.

3. Microfluidic Chip for cff DNA Extraction

Microfluidic chip design and fabrication protocol was developed in the laboratory of Dr. Anubhav Tripathi (Brown University, RI). Microfluidic chip fabrication protocol used in this

experiment was adapted from the one described in Cui et al. paper. [17] The microfluidic chip consists of polydimethylsiloxane (PDMS) body containing wells and channels covered by a glass slip. Single chip mold contains six T-shaped "units" which means six experiments can be conducted or repeated on one chip. Unused units can be stored for use later. This facet of the chip design maximizes efficiency as it eliminates the need of having to produce microfluidic chip for each experiment. Fabrication of one microfluidic chip takes about 2 hours, hence having more channels on one chip is more economical in terms of time.

Each T-shaped unit has three punctured wells and two intersecting channels. (**Figure 1**) Oil well (OW) is connected to one end of the short channel (SC) that bisects the long channel (LC). LC has two wells on each end. Sample well (SW) is on the left of the LC while elution well (EW) is on the right side of the LC. A 3.0 mm diameter whole punch was used to create each well. It is important to use a whole punch that is large enough to make a reservoir that can contain at least 10 μl of liquid sample. Moreover, channels must have appropriate width (1.25 mm) and depth (150 μm) for efficient transfer of magnetic beads. [17]

For this project, castor oil was inserted in the oil OW of the microfluidic chip. Through capillary action, castor oil moves along SC and spreads across LC. Just before the oil reaches the entrances of SW and EW, priming solution is pipetted into both SW and EW to create oil-water interfaces at both ends of LC. Then, sample suspension that contains magnetic beads will be loaded into SW of the chip. Using a magnet, hydrophobic molecules of the sample suspension (DNA bound to magnetic beads) will be able to break the oil-water interface and transported across LC towards EW, while hydrophilic molecules (buffer solutions) will remain in EW. The novelty of the device comes from the above-mentioned oil-water interface interacting with hydrophobic and

hydrophilic molecules of sample solutions. This simple device offers a quick and low-cost option for people who are looking for an efficient method of DNA extraction.

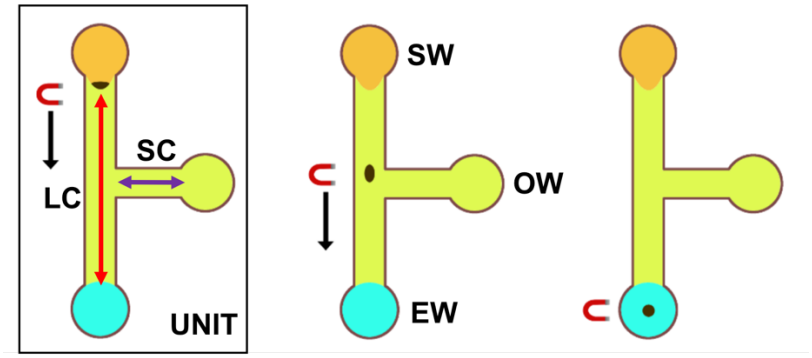

Figure 4. **Microfluidic chip channel design and operation.** Left: the silica-based magnetic bead and 200 bp DNA sample suspension is pipetted into the sample well (SW). An external magnetic separator is used to coalesce the suspension at the oil-water inter face. (Orange – hydrophilic TE solution, Green – castor oil, blue – hydrophilic elution buffer) Middle: An external magnetic separator is used to penetrate the oil phase and transfer the suspension across the long channel (LC). Right: The suspension is collected in the elution well (EW), and the contents are removed and vortexed for elution and downstream analysis. [17]

Materials & Methods

1. Microfluidic chip fabrication

Microfluidic chip fabrication protocol was developed by Dr. Tripathi and colleagues. [17] Using soft lithography, polydimethylsiloxane (PDMS) microfluidic chip that were used for on-chip experiments were produced. 10 g of PDMS precursor (Slygard® 184 Silicone Elastomer Base) (Dow Corning, Midland, MI) and 1 g of curing agent (Slygard® 184 Silicone Elastomer Curing Agent) (Dow Corning, Midland, MI) were mixed on a hexagonal polystyrene weighing dish. (Fisher Scientific International, Inc., Pittsburgh, PA) Upon mixing, the mixture was incubated in a vacuum chamber at 600 psi for 1 hour to remove air bubbles. Degassed mixture was poured into the microfluidic chip mold and was stored in 75 °C for 1 hour. Solidified chip in the mold was cut out with a razor blade. It is important to not disturb chip patterns during excision. The three wells of each channel were punctured with a 3 mm diameter hole-punch. The chip and glass cover (Pre-cleaned Gold Seal™ Microscope Slide, plain extra strength 1.2 mm thickness) (Thermo Fisher Scientific, Waltham, MA) were cleaned using tape first, then washed with methanol and dried with nitrogen gas. Before bonding the chip to a glass cover, the surface of the chip that touches the glass cover was oxygen plasma treated (PDC-32G Plasma Cleaner) (Harrick Plasma, Ithaca, NY). After bonding, microfluidic chips were ready to be primed. Alternatively, the chips could be stored in a 75 °C oven until priming. Priming solution master mix was prepared by mixing 425 µl of DI water, 50 µl of Tween® 20 (Sigma-Aldrich, St. Louis, MI), and 25 µl of Bovine Serum Albumin (BSA) (Sigma-Aldrich, St. Louis, MI). Primed chips were stored in the fridge for no more than two days prior to use.

2. On-chip DNA recovery in TE with carrier beads – Experiment 1 & 2

For on-chip DNA extraction in TE buffer (10 mM Tris-HCL, 0.1 mN EDTA) (Thermos Fisher Scientific, Waltham, MA) experiment, two different types of samples were prepared: (1) samples containing carrier beads (Agencourt AMPure XP) (Beckman Coulter Genomics, Danvers, MA) (2) samples made without carrier beads.

Master mix of samples containing carrier beads consisted of 1000 pg of NoLimits 200 bp DNA fragment (Thermos Fisher Scientific, Waltham, MA), 0.6 µl of MagSi DNA Allround (MagnaMedics, Geleen, Netherlands), and 0.6 µl Agencourt AMPure XP. (DNA serial dilution data in **[Appendix A]** and sample composition data in **[Appendix C]**) Volumetric ratio between MagSi DNA Allround and carrier beads was set to 1:1.

Master mix of samples without carrier beads were created by mixing 1000 pg of NoLimits 200 bp DNA fragment with 0.6 µl of MagSi DNA Allround. Upon addition, each master mix of samples were vortexed for 10 sec at 3000 rpm. Then, the master mix was incubated for 10 min in room temperature (RT). Pre-primed microfluidic chips were removed from the fridge (4 °C). Priming solution in both SW and EW of unit 1 was aspirated and replaced with 10 µl of sample solution and 10 µl of elution buffer (TE) respectively. A magnetic separator (New England BioLabs, Ipswich, MA) was put beneath the glass cover of the microfluidic chip. Moving around the separator right beneath SW of the chip in small circular motions, DNA bound magnetic beads were collected into a compact cluster in SW to facilitate the transport through the channel. When the sample in SW was densely packed into a small cluster, the magnet beneath the glass cover was dragged from SW to EW direction to break the oil water interface of SW and LC. Upon breaking the oil water interface, the sample was transferred to EW by slowly moving the magnet in the

direction of EW to minimize the "trailing effect" that leaves residues along LC. Transferred sample was collected with a pipette and stored in a test tube (Bio-Rad Laboratories, Inc., Hercules, CA) for further downstream quantification. The extraction process was repeated for unit 2 and unit 3. Total of 30 μl of master mix was used in three separate units of a microfluidic chip.

3. On-chip DNA recovery in human blood serum – Experiment 3

For on-chip 200 bp DNA extraction in human blood serum (experiment 3), protocol from experiment 1 was modified. In this experiment, Seratrol™ Off the Clot Human Serum Double Charcoal Stripped Delipidized (Golden West Biologicals, INC.®, Temecula, CA) was spiked with 200 bp DNA. **[Appendix B]** Master mix solution contained 1000 pg of 200 bp DNA and 0.6 μl of MagSi Allround DNA. After sample and blank master mix solutions were prepared, they were vortexed for 10 sec at 3000 rpm and incubated for 10 min in RT. Sample loading, on-chip transfer, and sample elution were conducted in three separate units. Methods were the same from experiment 1 and experiment 2.

4. Data Labeling

In this project, three experiments were conducted. Samples from each experiment were labeled as follow: sample X.Y.Z. 'X' represents experiment number, 'Y' represents sample solution type (i.e. with carrier beads or without carrier beads), and 'Z' represents unit number)

5. Quantification of DNA

Quantification of DNA was performed using Quant-iT™ PicoGreen dsDNA Assay Kit. (Life Technologies, Carlsbad, CA) PicoGreen® dye was diluted following the protocol provided by the manufacturer. Blank solution was prepared for each type of sample. Sample recovered from each unit was measured three times by aliquoting the recovered sample into three wells of the 96 well microplate (triplicate reading). Each sample fluorescence value was subtracted by corresponding blank fluorescence value. Standard curve was generated, and fluorescence signal obtained from results were converted to amount of DNA recovered using the standard curve equation. Amount of DNA [pg] obtained from triplicate readings of each unit was averaged to calculate the average amount of DNA extracted from that unit. Amount of DNA [pg] obtained from multiple unit was averaged to find the average amount of DNA recovered from each sample type.

6. Statistical Analysis

Means are reported and variance reported in this study represent standard error of means. T-test analysis was used for experiment 2 to assess AMPure XP bead effectiveness in enhancing DNA recovery and a P-value of <0.05 was considered significant.

Results

1. Experiment 1 – Recovery of DNA sample prepared in TE with MagSi Allround

The first on-chip DNA recovery experiment (experiment 1) was conducted to evaluate the possibility of short-fragmented DNA extraction on a novel microfluidic chip designed by the Tripathi lab. 200 bp DNA was diluted in TE buffer. For preliminary experiments, TE was used when preparing DNA samples as it prevents degradation of DNA. Standard curve was produced using lambda DNA. Average amount of DNA loaded on SW was 314.465 pg **[Appendix C]** Sample 1 was recovered from three units of a single microfluidic chip (10 µl per unit). Sample 1.1.1 represents experiment 1, sample 1 solution recovered from unit 1, and sample 1.1.2 represents experiment 1, sample 1 solution recovered from unit 2. **Figure 2** shows that 291.095 pg (± 27.788) of DNA was extracted from unit 1. 71.364 pg (± 22.688) of DNA was extracted from unit 2. On average, 181.229 pg (±109.866) of 200 bp DNA was recovered from sample 1 of experiment 1 which corresponds to average of 57% recovery. Sample 1.1.3, which was recovered from unit 3, was excluded from calculations for its uncertainties in fluorescence signal as the values of triplicates reached maximum value possible (260000 RFU).

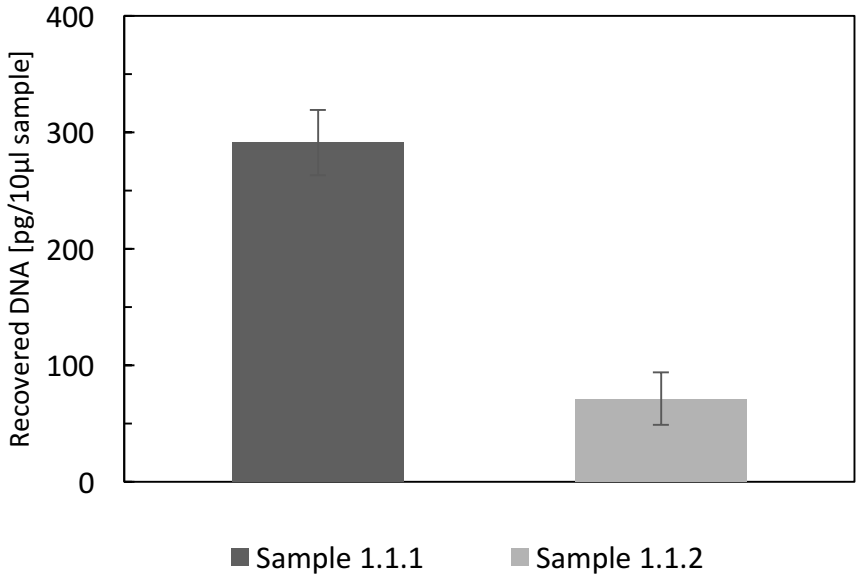

Figure 5. Experiment 1: Sample 1 (200 bp DNA prepared in TE with MagSi Allround) was recovered from unit 1 and unit 2. Amount of DNA recovered from unit 1 is represented as sample 1.1.1. Amount of DNA recovered from unit 2 is represented as sample 1.1.2.

2. Experiment 2 – Recovery of DNA sample prepared in TE with MagSi Allround and AMPure XP (Carrier bead effectiveness experiment)

Second on-chip experiment was conducted to assess effectiveness of carrier beads in short-fragmented DNA extraction. Sample 1 of experiment 2 contained 200 bp DNA, Mag-Si DNA Allround, lysis/binding buffer, and TE. Sample 2 in experiment 2 was prepared the same way as sample 1 with the addition of Agencourt Ampure XP. **[Appendix C]**

Experiment 2, sample 1 was recovered in three different units of a single microfluidic chip. Sample 2.1.1 represents experiment 2, sample 1 solution recovered from unit 1. Sample 2.1.3 represents experiment 2, sample 1 solution recovered from unit 3. Sample 2.1.2, which was recovered from unit 2 of the microfluidic chip, was excluded from calculations to prevent any distortions in analysis since its triplicate fluorescence values reached saturation. 14.946 pg (± 43.893) of 200 bp DNA was extracted from sample 2.1.1. On the other hand, 170.768 pg (±4.666)

of DNA was extracted and recovered from unit 3 of the microfluidic chip (sample 2.1.3). (**Figure 3A**) Average amount of DNA recovered from sample 1 was 92.857 pg (± 77.911) which gave it a 30.086 % DNA recovery. Amount of DNA loaded onto SW was 314.465 pg. **[Appendix C]**

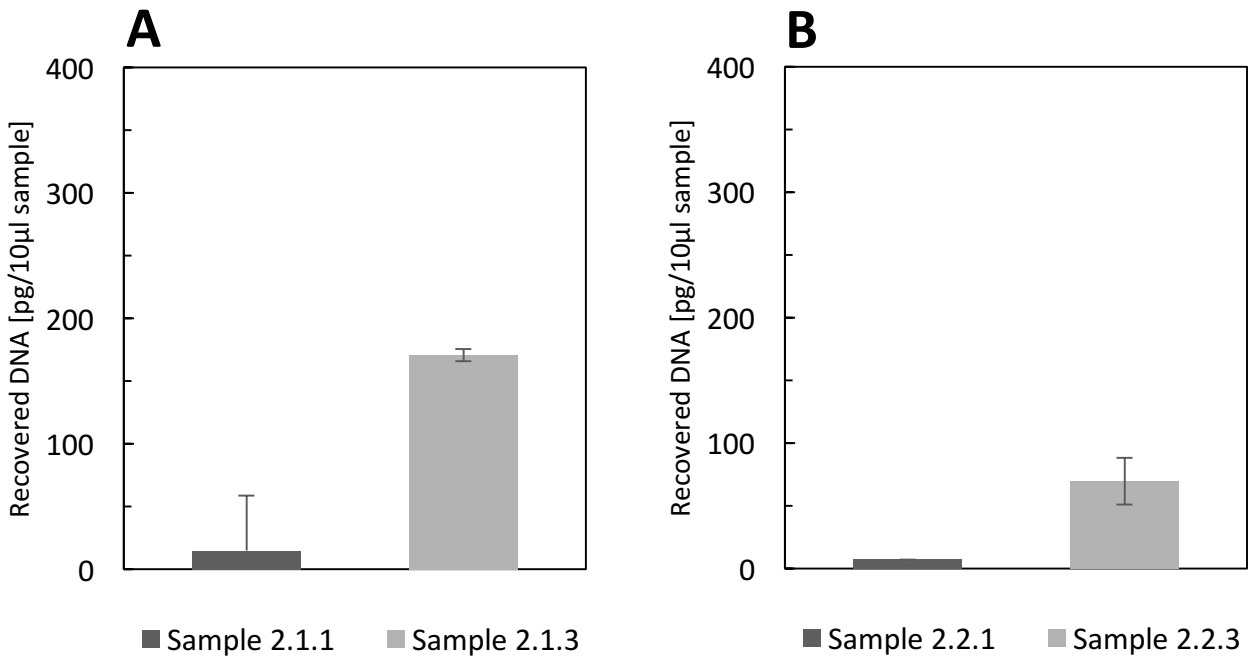

Figure 6. A) Experiment 2: Sample 1 (200 bp DNA prepared in TE with MagSi Allround beads) was recovered from unit 1 and unit 3. Amount of DNA recovered from unit 1 is represented as sample 2.1.1. Amount of DNA recovered from unit 3 is represented as sample 2.1.3. **B) Experiment 2: Sample 2 (200 bp DNA prepared in TE with MagSi Allround beads and AMPure XP beads) was recovered from unit 1 and unit 3.** Amount of DNA recovered from unit 1 is represented as sample 2.2.1. Amount of DNA recovered from unit 3 is represented as sample 2.2.3.

Sample 2 of experiment 2 contained carrier beads (Agencourt AMPure XP), thus corresponding blank solution was prepared. Just like sample 1, 10 µl of sample 2 solution was recovered from 3 different units of a microfluidic chip. **Figure 3B** shows that 7.376 pg of DNA was extracted from unit 1 (sample 2.2.1). Only one fluorescence value from sample 2.2.1 triplicate readings was

available since the other two fluorescence values reported maximum value errors. 69.834 pg (± 18.626) of DNA was extracted from unit 3 (sample 2.2.3). Average amount of DNA recovered from sample 2 was 38.605 pg (± 31.229). Input DNA was 308.642 pg. Hence, percentage recovery of average amount of DNA extracted with sample 2 was 12.508 %, which on average was less than half of that for non-carrier bead control ($P>0.05$, not significant).

3. Experiment 3 – Recovery of DNA sample prepared in human blood serum with MagSi Allround

Experiment 3 was conducted by spiking human blood serum with 200 bp DNA to replicate real world clinical environment where cff DNA is extracted from maternal blood serum or plasma. For comparison, sample 1 of experiment 3 was prepared and extracted in TE solution. 200 bp DNA, TE, MagSi DNA Allround, and L/B buffer were used for sample 1. **[Appendix C]** 16.288 pg (± 8.491) of 200 bp DNA was recovered from sample 3.1.1, 85.398 pg (± 6.066) was recovered from sample 3.1.2, and 84.838 pg (± 7.334) was recovered from sample 3.1.3 (**Figure 4**). On average, 62.175 pg (± 22.944) of 200 bp DNA was recovered from sample 1 of experiment 3. With the input amount of 314.465 pg of 200 bp DNA **[Appendix C],** sample 1 of experiment 3 had a 19.772 % recovery on average.

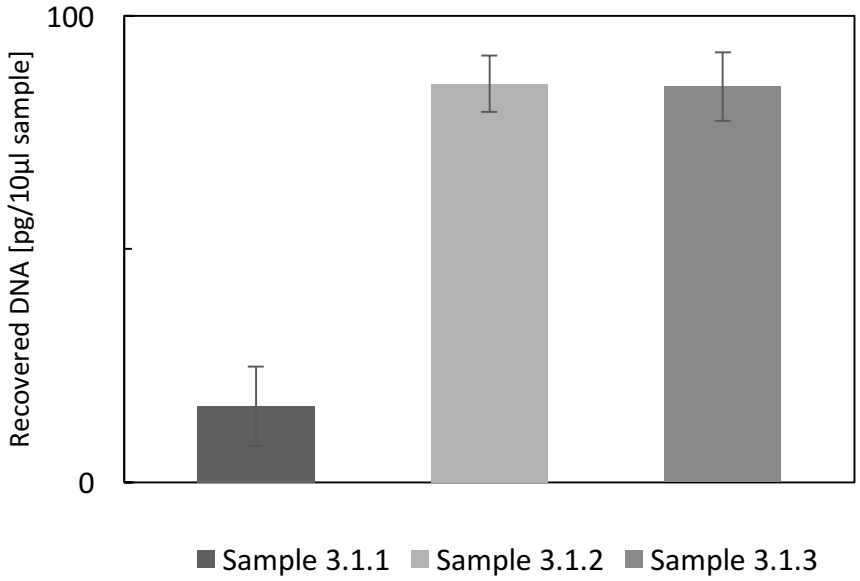

Figure 4. Experiment 3: Sample 1 (200 bp DNA prepared in TE with MagSi Allround beads) was recovered from unit 1, unit 2, and unit 3. Amount of DNA recovered from unit 1 is represented as sample 3.1.1. Amount of DNA recovered from unit 2 is represented as sample 3.1.2. Amount of DNA recovered from unit 3 is represented as sample 3.1.3.

Figure 5 shows the recovery data of experiment 3 sample 2. Sample 2 (DNA diluted in human blood serum) of experiment 3 was conducted and analyzed the same way as sample 1 of experiment 3. Sample 2 of experiment 3 contained 200 bp DNA, human blood serum, MagSi DNA Allround, and L/B buffer **[Appendix C]**. Each sample fluorescence value was subtracted by blank fluorescence value. 224.376 pg (± 2.726) of 200 bp DNA was recovered from sample 3.2.1. 164.778 pg (± 18.878) of 200 bp DNA was recovered from sample 3.2.2, and 69.134 pg (± 10.421) of 200 bp DNA was recovered from sample 3.2.3. On average, 152.763 pg (± 45.215) of 200 bp was recovered from sample 2 of experiment 3. As 314.465 pg of 200 bp DNA was loaded onto SW **[Appendix C]**, 48.579 % of the input was recovered from sample 2 of experiment 3, which is more than twice the recovery in the absence of human blood serum.

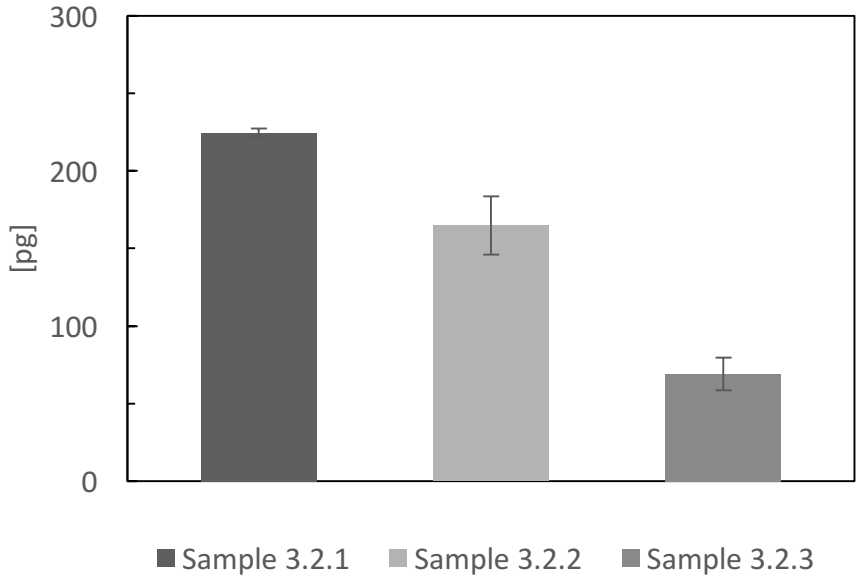

Figure 5. Experiment 3: Sample 2 (200 bp DNA prepared in human blood serum with MagSi Allround beads) was recovered from unit 1, unit 2, and unit 3. Amount of DNA recovered from unit 1 is represented as sample 3.2.1. Amount of DNA recovered from unit 2 is represented as sample 3.2.2. Amount of DNA recovered from unit 3 is represented as sample 3.2.3.

Discussion

The objectives of this study were to: (1) conduct preliminary 200 bp DNA recovery experiment in TE buffer on an original microfluidic chip designed by Dr. Tripathi and colleagues to learn the fundamental system of DNA extraction on-chip; (2) evaluate effectiveness of carrier beads when used in on-chip DNA recovery experiments with volumetric ratio of 1:1 with MagSi DNA Allround; (3) replicate real world non-invasive prenatal diagnostics by spiking human blood serum with 200 bp DNA and assess feasibility of future applications using the microfluidic chip. 200 bp DNA was diluted in TE first and was extracted using surface functionalized magnetic beads. Carrier beads were added to sample composition for experiment 2 to assess their effectiveness in

on-chip DNA recovery. Lastly, 200 bp DNA was diluted in human blood serum and was extracted on-chip using surface-functionalized magnetic beads without carrier beads.

1. **Experiment 1: DNA prepared in TE with MagSi Allround beads was successfully recovered**

There are many studies investigating the effectiveness of commercial DNA extraction kits using patient samples of maternal blood serum.[16] We wanted to validate our hypothesis that short-fragmented DNA bound to surface-functionalized magnetic beads can be recovered on our laboratory's novel microfluidic chip which takes advantage of the hydrophilic/hydrophobic interactions between samples and channel liquids. 200 bp DNA were used to replicate cff DNA for their similarities in base-pair length. DNA degradation preventive TE solution was used to dilute 200 bp DNA instead of human blood serum. The major issue repeatedly encountered during on-chip experiments was fluorescence response reaching maximum value possible at 260000 RFU, which required repetitions of experiments to produce analyzable data. Fluorescence data of triplicates that reached maximum value was excluded from calculations throughout the project to minimize distortions in analysis since they bore uncertainties. However, average fluorescence values obtained from triplicate data of unit 1 was 247002.667 RFU. This result implies the possibility of unit 3 triplicate fluorescence values being very close to the saturation point rather than exceeding it by significant amount. Hence, exclusion of data points that reached the saturation point does not imply more accurate result; it indicates consistency in data analysis. Results from several on-chip experiments carried out were discarded for the reason mentioned above. To resolve the issue of maximum fluorescence response, standard curve DNA range was adjusted from 0.05—90.0 pg to 1.389—150.0 pg for experiment 1 of this project. Although this adjustment produced

usable data for sample 1.1.1 and sample 1.1.2, sample 1.1.3 data was excluded for the same issue of maximum fluorescence signal. Using fluorescence data from sample 1.1.1 and 1.1.2, average yield of 181.299 pg (± 109.866) was achieved.

The disparity between sample 1.1.1 yield (291.095 pg) and sample 1.1.2 yield (71.363 pg) could be the result of inconsistencies in transport procedure from SW to EW using a magnetic separator. For samples with low yields, we experienced difficulties in forming a wholesome cluster of the sample suspension in SW of the chip which made the transport process more difficult. Low yield was expected for samples with visible cluster residuals left in SW upon completion of cluster transportation from SW to EW. Even when a compact and wholesome cluster could be formed, magnetic bead residues were visibly left behind along LC from trailing effect, which negatively affected recovery rate. To minimize trailing effect, optimization of volume of magnetic beads used in relation to channel size and/or the amount of DNA present in samples will be necessary.

Finally, a large standard error value (± 22.688) of sample 1.1.2 represents disparities in DNA yield among the triplicate data. The percentage difference in lowest yield triplicate data (38.177 pg) and highest yield triplicate data (114.756 pg) of sample 1.1.2 was 100.15 %. Two factors could have caused the disparity: (1) extracted sample not being thoroughly mixed prior to PicoGreen® assay (2) high fluorescence signal of blank solution.

2. Experiment 2: Sample that contained AMPure XP beads had lower DNA recovery on average

Carrier beads are paramagnetic beads that cause immobilization of DNA strands on their surface for isolation and extraction purposes. [33] Carrier beads in this experiment used were Agencourt AMPure XP. Carrier beads were added to the sample mixture in order to observe its

effectiveness in fortifying the 200 bp DNA yield on-chip. The volumetric ratio was set to 1:1 between MagSi DNA Allround and AMPure XP. Only one ratio is assessed in this experiment, however, it is highly desired to test the effect of the volumetric ratio on DNA recovery in future experiments.

Sample 2.1.Z represents experiment 2 sample mixture that does not contain carrier beads, recovered from unit Z. Fluorescence data collected from sample 2.1.2 was excluded from yield calculations as it experienced max fluorescence issue. Sample 2.2.Z represents on-chip experiment 2 sample mixture that contained carrier beads, recovered from unit Z. For sample 2.2.1, only one data among the triplicate readings (7.376 pg) was usable as the other two values reached the maximum fluorescence signal. As mentioned before, exclusion of fluorescence values that reached saturation point remained consistent through out data analysis. Lack of extra data requires repetition of the experiment in the future. Sample 2.2.2 was excluded from yield calculations due to max fluorescence signal issue. Sample 2.2.3 had positive yield (32.583 pg, 88.118 pg, and 88.801 pg), however, percentage difference between its lowest triplicate data and the highest triplicate data was 92.628 %. To produce more consistent yield from triplicates, the eluted sample must be mixed thoroughly prior to PicoGreen® assay. Moreover, large standard error of the data was observed when DNA yield from sample 2.2.1 and sample 2.2.3 were averaged.

Average percentage yield of sample 2.1 that did not contain carrier beads (30.086 %) and sample 2.2 that contained carrier beads (12.508 %) show that carrier beads were not effective in increasing DNA yield. Statistical analysis showed that there is no statistical difference between the two samples ($P>0.05$) when volumetric ratio was set to 1:1 (MagSi Allround : AMPure XP). Carrier bead experiment of this project was preliminary, had technical difficulties that limited sample size, and requires further investigation of buffer solutions and volumetric ratios in order

draw a more definitive conclusion of the effect of carrier beads. Studies suggest that addition of optimal amount of salts and polyethylene glycol (PEG) affects carrier bead's performance. [34], [35] Future on-chip experiments exploring carrier bead effectiveness could involve optimization of buffer solution with different ratios of Na^+ salts, PEG, and carrier beads.

3. Experiment 3: DNA sample that was prepared in human blood serum produced higher yield than sample prepared in TE

There are many commercial DNA extraction kits in the market that provides ready-to-use reagents for traditional laboratory settings. [20], [36] The microfluidic chip developed by Dr. Tripathi and colleagues offers a simple, low-cost, and effective system for point-of-care DNA extraction application. In experiment 3, real world clinical application of cff DNA was replicated by spiking human blood serum with 200 bp DNA. Along with the serum sample mixture, 200 bp DNA was prepared in TE, following the sample preparation protocol used in experiment 1 and experiment 2, for comparison. The sample solution was used to observe feasibility of DNA recovery on the Tripathi microfluidic chip and to establish fundamental protocol for short-fragmented DNA extraction in serum.

Sample 3.1.Z represents 200 bp DNA diluted in TE. Maximum fluorescence value problem was not encountered in experiment 3, hence data obtained from all three units were used in average yield calculations. On average, 62.175 pg (± 22.944) was recovered from sample 3.1.Z. Percentage yield was 19.772 %. In sample 3.1.1 data, negative fluorescence data was observed for the readings of unit 1 triplicates which resulted in low yield on average. High fluorescence signal of the blank solution as well as uneven suspension of the mixture in the eluted sample could have generated low yield. Sample 3.2.Z represents 200 bp DNA diluted in human blood serum. In other words,

human blood serum was spiked with 200 bp DNA. No maximum fluorescence signal issue was experienced with sample 3.2.Z. Average yield of sample 3.2.Z was 152.763 pg (± 45.215). Percentage recovery was 48.579 %. Compared to the yield of sample 3.1.Z which was prepared in TE, sample 3.2.Z that was prepared in human blood serum produced higher yield. This could be result of presence of various proteins (especially albumin) in human blood serum. Serum albumin could have inhibited non-specific binding of DNA molecules to the polymer surface of the microfluidic chip, which could have resulted in increased amount of specific binding between magnetic beads and DNA molecules.

Results show that short-fragmented DNA recovery study using human blood serum is feasible with the microfluidic chip designed by the Tripathi lab. Future serum experiment suggestions include: (1) yield comparison study involving commercial DNA extraction kits currently available on the market, and (2) size distribution study utilizing the size-selective property of AMPure XP in solution that contains 200 bp DNA, 1000 bp DNA, and human blood serum. AMPure XP beads are known to be able to selectively extract DNA strands of a certain base pair length when optimized conditions are provided according to the manufactuerer. 200 bp DNA can substitute for cff DNA while 1000 bp can substitute for cell free maternal DNA. Successful implementation of the experiment will bring the Tripathi lab designed microfluidic chip one step closer to point-of-care application.

4. Limitations and Future Directions

One of the limitations of this study include lack of positive controls. Amount of DNA present in the initial samples should have been measured with PicoGreen® assay for thorough

analysis of results. Moreover, for carrier bead effectiveness experiment (experiment 2), a third sample that consists of DNA and AMPure XP beads should have been tested for comparison.

There are a few suggestions in terms of microfluidic chip fabrication protocol that could be conducive to future experiments. While conducting various experiments throughout the project, the magnetic bead and DNA suspension was observed adhering to the glass surface of the device. Although the protocol states that the device is ready to be primed right after bonding, device storage in the 75 °C oven for at least 48 hours is recommended to avoid surface adhesion problems. Additionally, an anti-static gun can be adapted in the protocol to assess its effectiveness in preventing surface adhesion.

To minimize the trailing effect and facilitate sample transfer along LC, it is necessary to optimize the number of magnetic beads used in experiments. Excessive amount of magnetic beads used could decrease the yield by 1) clogging LC thus leaving residues along the channel and 2) causing the beads to stick to each other which reduces the total surface area for DNA binding. Comparison of DNA recovery data with varying ratios between concentrations of magnetic beads and 200 bp DNA is important to further improve DNA recovery yield in future experiments.

Conclusions

This project demonstrated feasibility of isolating and recovering short-fragmented DNA from human blood samples with a novel microfluidic chip technology. This project was motivated by current field interest in extraction of cell-free fetal DNA from maternal blood serum or plasma in an attempt to develop a more efficient non-invasive prenatal diagnostic modality. 200 bp DNA, a substitute of cell-free fetal DNA, was successfully recovered from human blood serum samples on a microfluidic chip using surface-functionalized magnetic beads and an external magnetic separator to draw samples through an oil interface for separation. The system offers effectiveness, rapidity, and ease of operation compared to commercial DNA extraction kits currently available in the market. With further investigations involving size-selectivity as well as compatibility with downstream applications such as PCR, the technology could serve as a promising option for both patients and healthcare providers whom desire more accurate and efficient non-invasive prenatal diagnostics.

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Appendix

[Appendix A] 200 bp DNA dilution

200 bp DNA diluted in TE: DNA serial dilution table for experiment 1, experiment 2.1, and 3.1 is presented in **Table 1**. Stock concentration of 200 bp DNA was 500000 pg/µl. 1 µl of stock solution of 200 bp was added to a 500 µl test tube that contained 99 µl TE (dilution 1), creating 5000 pg/µl solution. 2 µl of the 5000 pg/µl solution was added to a 500 µl test tube that contained 98 µl of TE. Final concentration of 200 bp DNA was 100 pg/µl.

Table 1. 200 bp DNA diluted in TE for experiment 1, experiment 2.1, and experiment 3.1

Serial Dilution	Previous solution [µl]	TE [µl]	Total volume [µl]	concentration [pg/µl]
stock (200 bp DNA)	—	—	—	500000.000
dilution 1	1.000	99.000	100.000	5000.000
dilution 2	2.000	98.000	100.000	100.000

[Appendix B] 200 bp DNA dilution

200 bp DNA serial dilution table for experiment 3.2 is presented in **Table 2**. Stock concentration of 200 bp DNA was 500000 pg/µl. 1 µl of stock solution of 200 bp was added to a 500 µl test tube that contained 99 µl human blood serum (dilution 1), creating 5000 pg/µl solution. 2 µl of the 5000 pg/µl solution was added to a 500 µl test tube that contained 98 µl of human blood serum. Final concentration of 200 bp DNA was 100 pg/µl.

Table 2. 200 bp DNA diluted in TE or experiment 3.2

Serial Dilution	Previous solution [µl]	Serum [µl]	Total volume [µl]	concentration [pg/µl]
stock (200 bp DNA)	—	—	—	500000.000
dilution 1	1.000	99.000	100.000	5000.000
dilution 2	2.000	98.000	100.000	100.000

[Appendix C] Sample composition

Sample composition for experiment 1 is presented in **Table 3**. 10 µl of 200 bp DNA from dilution 2 of table 1 was added to the master mix solution that contained 0.6 µl of MagSi Allround magnetic beads and 21.2 µl of lysis/binding buffer (L/B buffer). Volumetric ratio between magnetic bead + DNA to L/B buffer was set to 1:2. 10 µl of master mix solution was loaded onto SW of each unit of the microfluidic chip, which translates to input DNA to 314.465 pg per unit.

Table 3. Sample composition table for experiment 1: 200 bp DNA diluted in TE

Master mix Composition (experiment 1)	DNA Concentration [pg/µl]	Volume [µl]
200 bp DNA dilution 2	100	10
Magnetic beads	-	0.60
L/B buffer	-	21.20
Master Mix	31.4465	31.80
DNA in sample volume (10 µl)	314.465	—

Sample composition for experiment 2.1 (without carrier beads) is presented in **Table 4**. 10 µl of 200 bp DNA from dilution 2 of table 1 was added to the master mix solution that contained 0.6 µl of MagSi Allround magnetic beads, 21.2 µl of lysis/binding buffer (L/B buffer), and 0.6 µl of TE. Volumetric ratio between magnetic bead + DNA to L/B buffer was set to 1:2. 10 µl of master mix solution was loaded onto SW of each unit of the microfluidic chip, which translates to input DNA to 308.642 pg per unit.

Table 4. Sample composition table for experiment 2.1 (without carrier beads): 200 bp DNA diluted in TE

Master Mix Composition (experiment 2.1)	Concentration [pg/µl]	Volume [µl]
200 bp DNA dilution 2	100	10
magnetic beads	-	0.60
L/B buffer	-	21.20
TE	-	0.60
Master Mix	30.86419	32.40
[pg] DNA in sample volume (10 µl)	308.642	-

Sample composition for experiment 2.2 (with carrier beads) is presented in **Table 5**. 10 µl of 200 bp DNA from dilution 2 of table 1 was added to the master mix solution that contained 0.6 µl of MagSi Allround magnetic beads, 21.2 µl of lysis/binding buffer (L/B buffer), and 0.6 µl of AMPure XP carrier beads. Volumetric ratio between magnetic bead + DNA to L/B buffer was set to 1:2. 10 µl of master mix solution was loaded onto SW of each unit of the microfluidic chip, which translates to input DNA to 308.642 pg per unit.

Table 5 Sample composition table for experiment 2.2 (with carrier beads): DNA diluted in TE

Master Mix Composition (experiment 2.2)	Concentration [pg/µl]	Volume [µl]
200 bp DNA dilution 2	100	10
magnetic beads	-	0.6
L/B buffer	-	21.2
carrier beads	-	0.6
Master Mix	30.86419753	32.4
[pg] DNA in sample volume (10 µl)	308.6419753	-

Sample composition for experiment 3.1 is presented in **Table 6**. For experiment 3.1, 10 µl of 200 bp DNA from dilution 2 of table 1 was added to the master mix solution that contained 0.6 µl of MagSi Allround magnetic beads and 21.2 µl of lysis/binding buffer (L/B buffer). Volumetric ratio between magnetic bead + DNA to L/B buffer was set to 1:2. 10 µl of master mix solution was loaded onto SW of each unit of the microfluidic chip, which translates to input DNA to 314.465 pg per unit.

Table 6. Sample composition table for experiment 3: DNA diluted in TE

Master mix Composition (in TE)	DNA Concentration [pg/µl]	Volume [µl]
200 bp DNA dilution 2	100.0	10.0
Magnetic beads	-	0.60
L/B buffer	-	21.20
Master Mix	31.44654	31.80
DNA in sample volume (10 µl)	314.46541	—

Sample composition for experiment 3.2 is presented in **Table 6**. For experiment 3.1, 10 µl of 200 bp DNA from dilution 2 of table 2 was added to the master mix solution that contained 0.6 µl of MagSi Allround magnetic beads and 21.2 µl of lysis/binding buffer (L/B buffer). Volumetric ratio between magnetic bead + DNA to L/B buffer was set to 1:2. 10 µl of master mix solution was loaded onto SW of each unit of the microfluidic chip, which translates to input DNA to 314.465 pg per unit.

Table 7. Sample composition table for experiment 3: DNA diluted in human blood serum

Master mix Composition (in TE)	DNA Concentration [pg/μl]	Volume [μl]
200 bp DNA dilution 2	100.00000	10.00000
Magnetic beads	-	0.60000
L/B buffer	-	21.20000
Master Mix	31.44654	31.80000
DNA in sample volume (10 μl)	314.46541	—