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Topography-Assisted Electromagnetic Platform for Blood-to-PCR in a Droplet

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Abstract

This paper presents an electromagnetically actuated platform for automated sample preparation and detection of nucleic acids. The proposed platform integrates nucleic acid extraction using silica-coated magnetic particles with real-time polymerase chain reaction (PCR) on a single cartridge. Extraction of genomic material was automated by manipulating magnetic particles in droplets using a series of planar coil electromagnets assisted by topographical features, enabling efficient fluidic processing over a variety of buffers and reagents. The functionality of the platform was demonstrated by performing nucleic acid extraction from whole blood, followed by real-time PCR detection of KRAS oncogene. Automated sample processing from whole blood to PCR-ready droplet was performed in 15 minutes. We took a modular approach of decoupling the modules of magnetic manipulation and optical detection from the device itself, enabling a low-complexity cartridge that operates in tandem with simple external instruments.

Keywords

Micro-total-analysis system; solid-phase DNA extraction; droplet manipulation; real-time PCR

Nucleic acids-based diagnostics is a fast-growing field encompassing many applications including infectious diseases, oncology, pharmacogenomics and genetic testing. Microfluidic technologies used to create miniaturized total analysis systems (μ TAS) have demonstrated the potential to move nucleic acids-based diagnostic tools to the front lines of health care in the last two decades (Mark et al., 2010). Several examples of continuous flow microfluidic technologies have already demonstrated the ability to carry out nucleic acid extraction, amplification and detection of genetic biomarkers on a single platform (Burns et al., 1998, Easley et al., 2006, Lien et al., 2007, Pal et al., 2005, Sauer-Budge et al., 2009 and Zhang et al., 2010). Nevertheless, several challenges remain before these platforms can be

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adopted in clinical setting (Mariella, Jr, 2008 and Park et al., 2011). One often-cited problem is the difficulty of interfacing real-world biological samples with microfluidic platforms. Another major challenge is the reliance on supporting instruments that are not amenable to scaling down for the use outside of laboratory, which inhibits many technologies from advancing beyond the proof-of-concept stage. In light of these challenges, droplet-based platforms using droplets for storage and processing of microliterscale reagents have demonstrated the potential to bring μ TAS closer to clinics. Sample routing in these platforms are typically pumpless and valveless, providing ease in integration and greater flexibility to handle various physiological samples and assays (Abdelgawad and Wheeler, 2009 and Pollack et al., 2011).

Droplet-based microfluidic devices using dielectrophoretic (Fan et al., 2009, Hunt et al., 2008 and Velev et al., 2003), electrowetting (Boles et al., 2011, Fan et al., 2009, Pollack et al., 2011, Sista et al., 2008, Srinivasan et al., 2004 and Welch et al., 2011), optical (Chiou et al., 2003 and Park et al., 2009), and magnetic (Ali-Cherif et al., 2012, Berry et al., 2011, Lehmann et al., 2006, Pipper et al., 2007, Rida et al., 2003, Shikida et al., 2006, Tsuchiya et al., 2008 and Zhang et al., 2011) actuation mechanisms have made significant progress towards realizing essential manipulation for bioanalysis such as transport, mixing, splitting, and merging of discrete droplets. In particular, magnetic particle-based droplet manipulation is readily compatible with biomolecule extraction from crude samples using various commercially available reagents and surface-functionalized particles, providing an ease of integration over purely dielectrophoretic or electrowetting modes of operation (Pollack et al., 2011). Using this advantage, several magnetic particle-based platforms have demonstrated DNA extraction and purification on chip using silica-coated magnetic beads (Berry et al., 2011, Lehmann et al., 2006, Pan et al., 2011, Pipper et al., 2007 and Zhang et al., 2011). Movable permanent magnets have been used to successfully perform transport, splitting, merging of droplet for genetic assay (Pipper et al., 2007, Tsuchiya et al., 2008 and Zhang et al., 2011). However, automation of droplet processing with these methods requires the use of costly translational stages. Moreover, permanent magnet-actuated platforms generally lack strategies for bead agitation, which may substantially decrease the binding/washing efficiency (Zhang et al., 2011). Addressing these limitations, planar coils have been demonstrated to be capable of generating effective magnetic fields for long-range transport and manipulations of droplets (Beyzavi and Nguyen, 2009 and Rida et al., 2003), providing a simplified apparatus for droplet operations.

Among all droplet manipulation steps, splitting of magnetic particles from the parent droplet presents the greatest challenge because it depends on multiple factors including capillary force, magnetic particle load, and magnet velocity (Long et al., 2009). Droplet immobilization provides a simpler approach to this challenge. Successful splitting is often contingent on effective immobilization of parent droplet, and multiple strategies have been discussed. Typical strategies include substrate patterning (Lehmann et al., 2006 and Pipper et al., 2007) or placing physical barriers (Shikida et al., 2006 and Zhang et al., 2011). The first strategy involves local patterning of hydrophilic spots in order to anchor the aqueous droplets on a flat surface. However, such patterns also have affinity towards biomolecules and particles, resulting in adsorption of sample molecules as well as increased friction on magnetic particles. Following the alternative strategy, we recently reported a surface topography assisted droplet splitting based on a permanent magnet platform, where the elevated structures on chip improved the robustness of dissociating magnetic particles from the parent droplets (Zhang et al., 2011).

In this study, as illustrated in Scheme 1, we propose an electromagnetic droplet platform for PCR-based genetic testing in which magnetic particles are actuated using coil array-induced magnetic field gradients and are assisted by topographical barriers for splitting operations.

The combination of electromagnetic coils and topographical barriers enables efficient and automated manipulations of droplets from a wide variety of buffers and reagents – including lysis, binding, wash, elution buffer and PCR reagent – required for carrying out the entire process of genetic detection assays. We demonstrate the functions of our droplet manipulation platform by performing all processing steps between whole blood sample input and real-time amplification detection on a single cartridge.

2. Material and Methods

2.1 Fabrication of the droplet cartridge

The cartridge consisted of seven compartments connected serially by six sieve structures (see Scheme 1). Each sieve was designed with a radius of curvature of 1.5 mm and a gap width of 750 μm . Outer dimensions of the cartridge were approximately 56 $\times$ 15 $\times$ 6 mm. Acrylonitrile butadiene styrene (ABS) molds were generated by 3D rapid prototyping (Dimension 1200es, Breakaway Support Technology, USA). Polydimethylsiloxane, (Sylgard 184, Dow Corning Corp., USA) prepared with base-to-crosslinker ratio of 9:1, was cast into the mold and cured at 80°C for 30 minutes. The demolded structure was covalently bonded to a glass cover slip with a thickness of 100 μm (GOLD SEAL cover glass, Electron Microscopy Sciences, USA) following standard O₂ plasma treatment process. The device was subsequently dip-coated in a 1% w/w Teflon AF 1600 (DuPont Corp., USA) in FC-40 solvent (3M Company, USA) and baked overnight at 80°C. Teflon coating was applied in order to protect against surface adsorption of biomolecules and to prevent spreading of reagent droplets on the cartridge surface. The completed prototype is shown in Scheme 1A.

2.2 Magnetic bead manipulation system

Scheme 1B depicts the schematic assembly of the magnetic bead manipulation system. The fabricated device was attached on top of PCB containing a linear array of planar coils that were used as electromagnets to manipulate the magnetic beads. The sieve structures were aligned to the coils as shown in Scheme 1C to ensure proper droplet manipulation. The magnitude of coil-induced magnetic fields was controlled by modulating the amplitude of driving current source, while the directions of fields generated by each coil were controlled by an H-bridge circuit. Custom software written in LabVIEW (National Instruments, USA) was used to sequentially control the magnitude, duration and direction of magnetic fields. Automated operations were achieved by programming a complete routine for particle splitting and agitation operations in each reagent for DNA extraction and PCR.

A set of permanent magnets were placed on top of a soft magnetic steel plate, generating a 50 $\times$ 10 mm plane of uniform transverse magnetic field measuring approximately 50 mT. Magnetic field gradients were adjusted by a two-layer, 200 μm thick PCB. The PCB contained 7 coils on the top layer and 6 coils on the bottom layers, in which there was a partial overlap of adjacent coils with the center-to-center distance of 3 mm. Each coil was designed in a square profile with 8 windings. The height, width, and pitch of the copper lines were 35 μm , 150 μm , and 150 μm , respectively.

A thermoelectric module (Custom Thermoelectric Inc, USA) was placed underneath the PCB as a cooling pad to alleviate the effects of Joule heating on thermally sensitive reagents such as the PCR mixture, which contains heat-activated enzymes. Cooling temperature was feedback controlled by using a commercial PID controller (Accuthermo Technology, USA) and a K-type thermocouple (Omega Engineering Inc, USA) mounted on the surface of the module.

2.3 Cartridge priming and assay protocol

The on-chip procedure for the cell lysis, extraction and purification of nucleic acids followed the standard protocol based on solid phase extraction methods using commercially available silica-coated magnetic particles. Magnetic particles and reagents for nucleic acid extraction were purchased from Roche (MagnaPure LC DNA Isolation Kit I, Roche Diagnostic Corporation, USA). Unspun human whole blood from male donor containing heparin anti-coagulant was purchased from Biological Specialty Corporation (Colmar, PA, USA). The cartridge described previously was first filled with mineral oil (M5904, Sigma-Aldrich, USA) containing 0.5% w/w of surfactant Span-80 (Sigma-Aldrich, USA) to prevent the evaporation of reagents. Sessile reagent droplets (10-30 μ L) corresponding to each step of the assay protocol were loaded sequentially into the compartments. 5 μ L of whole blood was transferred into a mixture containing 10 μ L lysis/binding buffer (LSB), 5 μ L Tris-EDTA buffer, 1 μ L Proteinase K (20 mM) and 3 μ L of magnetic particles, and the mixture was dispensed into the first compartment of the device. Compartments 2 through 6 were sequentially loaded with the following reagents: 15 μ L washing buffer 1 (WB1a), 15 μ L washing buffer 1 (WB1b), 15 μ L washing buffer 2 (WB2a), 15 μ L washing buffer 2 (WB2b), and 10 μ L PCR reagent mixture, as illustrated in Scheme 1D. The lysis mixture in the first compartment was incubated on chip for 5 minutes with 100 cycles of agitation. Each washing step consisted of merging particle with the wash buffer, 15 cycles of mixing/agitation for 45 seconds, and splitting of particle into the next reagent droplet. Lastly, genomic DNA was eluted into the PCR mixture droplet by incubating the magnetic particles for 5 minutes with continuous agitation. The last compartment was intentionally left empty as a waste collection reservoir. PCR reagent mixture was prepared using QIAGEN HotStarTaq Core Kit (QIAGEN N.V., Netherlands). Total time required for electromagnet-actuated sample preparation was 15 minutes including all incubation steps, only a fraction of time required for manual extraction by a technician (Dundas et al., 2008).

2.4 Nucleic acid amplification and detection

After sample preparation was performed using the magnetic droplet manipulation system, the cartridge was transferred to a custom-built instrument for thermal cycling and fluorescence detection (Supplementary Scheme 1). Thermal cycling was performed using a thermoelectric module and PID controller in a similar arrangement used for Joule heat management. In order to account for transition times and temperature offsets between the surface of thermoelectric module and the droplet, thermal cycling conditions were calibrated to temperature profile obtained by monitoring a 10 μ L PCR mixture with a secondary thermocouple. Temperature profile obtained during calibration is presented in Supplementary Fig. 1. Primers targeting codon 12 of exon 2 of the KRAS oncogene was used as previously described (Ho et al., 2004). Primers were synthesized by Integrated DNA Technologies Inc. (USA) and 1 $\times$ LC Green Plus+ (Idaho Technologies Inc, USA) was used as the double-stranded DNA binding reporter dye. Primer sequences are presented in Supplementary Table 1.

Fluorescence was detected using a custom-built, portable optical instrument in epifluorescence configuration consisting of a blue light-emitting diode (LED) source ($\lambda_{\text{max}}=470\text{nm}$) and photodiode. Briefly, pulsed light from LED was passed through an excitation filter, reflected by a dichroic mirror, and was then focused onto the PCR reagent mixture droplet. Fluorescence emission from the reporter dye bound to genomic target was passed through an emission filter and focused onto a frequency-sensitive detector. The signals from the detector were recorded using analog-to-digital acquisition (USB-6229, National Instruments, USA) at a 5-second interval with a bin time of 150 ms. Signals obtained over the last 25 seconds of each annealing phase was averaged and plotted to generate a real-time amplification profile.

Agarose gel electrophoresis was performed to verify product amplified on droplet platform. Gels were run at 8 V/cm for 60 minutes. DNA was stained using GelStar nucleic acid gel stain (Lonza Rockland Inc, USA) and imaged under an epi-illumination configuration using a Kodak Gel Logic 200 Imaging System (Kodak, USA).

3. Results and Discussion

3.1 Operating procedure

In this droplet cartridge (Scheme 1D), genomic DNA extraction from whole blood and subsequent real-time amplification were integrated into a single process on the cartridge. First, whole blood sample was loaded to the initial compartment containing lysis buffer mixture and incubated. During this process, the white blood cells in sample matrix were lysed to release the genomic material. The released genomic DNA was adsorbed to silica-coated surface of magnetic particles in the presence of chaotropic salt in the lysis/binding buffer. After incubation, magnetic particles were split from the lysis buffer using coil-induced field gradient and merged into a series of washing buffers. The magnetic particles were then merged with the last droplet reagent containing PCR mixture and incubated. The lower concentration of salt present in a PCR mixture enables the genomic DNA bound on the particle surface to be released into the mixture. Afterwards, the magnetic particles were split from the PCR mixture into the waste chamber and the entire cartridge was loaded onto a custom-built thermal cycler and optical detection instrument for real-time amplification on chip.

3.2 Droplet manipulation platform

Droplet kinematic behaviors were governed by the interaction between two forces. The first was the magnetic force acting on the magnetic particles within the droplet, while the second was the capillary force induced by droplet deformation (Long et al., 2009). The proposed droplet manipulation strategy employed on-cartridge topographic features to control capillary forces, with an external actuation mechanism to control magnetic force on particles. Actuation of magnetic particles is realized on this platform using planar coil-induced magnetic field gradients in presence of uniform static field (Rida et al., 2003). Briefly, this mechanism utilizes a large, uniform transverse magnetic field B_0 to strongly polarize the magnetic moments of magnetic particles (Fig. 1A). Planar coils are used to induce small magnetic fields in parallel with B_0 . Since the magnetic force acting on each particle is described by the dot product of magnetic moment and the applied field gradient (Shevkoplyas et al., 2007), force is generated towards the direction where the transverse component (B_z) of field generated by planar coils is maximized. Controlling the direction of driving current allows switching of the polarity of coil-induced field, resulting in magnetic field gradient that can be made both positive and negative. This enables both attractive and repulsive forces which is necessary to perform transport, splitting and agitation of magnetic particles.

Meanwhile, capillary force is controlled using sieve structures to facilitate droplet splitting. Capillary force has two components, the Laplace force and the interfacial tension force (Chau et al., 2007). As illustrated in Fig. 1A, sieve structures were incorporated between reagent compartments to facilitate droplet deformation during splitting. When the magnetic droplet was actuated through the sieve, the droplet was deformed to attain negative mean curvature resulting in negative Laplace force. This phenomenon mitigates the effective capillary force, enabling droplet fission with a relatively low magnetic force.

3.3 Droplet splitting and merging

To split magnetic particles from the droplet with maximal retention of particles, a magnetic field gradient was generated by applying a positive, zero, and negative current to three consecutive coils, respectively (Fig. 1B). The magnetic fields were simulated numerically using commercial software (CFD-ACE+, CFD-RC, USA) and further characterized by measuring the coil-generated fields (Supplementary Fig. 2A and 2B). The resulting magnetic force was sufficient to overcome the capillary force and cause droplet fission. In order to mitigate the effects of Joule heating on interfacial tensions and thermally sensitive reagents such as the PCR mixture, the temperatures inside the droplet during the splitting process were maintained below 30°C by using a thermoelectric cooler located below the PCB. Joule heating is characterized and presented in Supplementary Fig. 2C.

Droplet splitting is primarily influenced by the interfacial tension between the droplet and the surrounding medium, as well as the magnetic force. In the proposed assay, we observed three levels of interfacial tensions between different reaction droplets and surrounding oil medium: 1) lysis mixture containing the lysis/binding buffer (LBS), which includes 20-30% w/w Triton X-100, 2) washing buffers 1 and 2 (WB1 and WB2) containing 30-60% w/w ethanol, and 3) the PCR master mixture mostly composed of water and salts. Experimental conditions were optimized such that consistent splitting and merging could be achieved in all three types of reagents. Splitting conditions were adjusted by varying three factors: 1) magnetic particle load, 2) surfactant concentration in surrounding oil medium, and 3) sieve gap. Topography-assisted splitting enabled us to apply a wide range of particle load to optimize the splitting conditions simultaneously for the reagents with several different interfacial tensions. If particle load was less than 200 µg, the magnetic force applied to the beads was insufficient to overcome interfacial tension of the parent droplet. With particle load in excess of 600 µg, the separated bead plug carried a substantial amount of buffer from the parent droplet. Particle loads between these limits were examined to qualitatively assess the splitting capability from various reagents. Higher surfactant concentration was observed to cause droplet instability indicated by difficulty in priming the device with reagents, while lower surfactant concentration was accompanied by increased difficulty in splitting. Variation in sieve gap size demonstrated poor repeatability in splitting operation with gap sizes smaller than 500 µm, due to the increased resistance to particle extraction from topographical barrier. Gaps larger than 1000 µm resulted in large amounts of buffer carryover, as the particles were unable to dissociate from the parent droplet. An intermediate gap size of 750 µm showed high repeatability with low carryover volume. At the optimal splitting condition, minimal to no residual particle was observed in the parent droplet.

Fig. 2 shows a photographic sequence of surface topography-assisted droplet splitting process, where a droplet containing magnetic particles is deformed through the sieve structures and dissociated into a small plug carrying trace amount of aqueous material from the parent droplet (Supplementary Video S1). As field maximum was generated at the destination compartment, magnetic particles were collected into a plug and pulled through the sieve structure until scission occurred at the elongated neck (Fig. 2D). After splitting from the parent droplet, magnetic particles were merged with the subsequent droplet. At the optimal concentration of surfactants, magnetic particle plug could be brought into close proximity with subsequent droplet without immediate merging upon contact. The resulting conditions enabled the droplet compartments to be packed more compactly on a smaller cartridge footprint. Droplet merging could be actively induced by generating field maximum at the center of current compartment, resulting in merger of the magnetic plug with the target droplet and collection of magnetic particles at the center of the droplet (Fig. 2E).

3.4 Particle agitation

One of the advantages of this platform is that efficient mixing can be achieved by agitating magnetic particles under alternating attraction and repulsion forces, as illustrated in Fig. 1C. Particle agitation involves a single coil centered on the droplet. When the direction of coil-generated magnetic field is parallel to background field, the maximal field occurs at the center of coil and particles are concentrated at the center of droplet. When the current is reversed, coil-generated field is antiparallel to background field; the center of droplet becomes a local field minimum, and particles are dispersed towards the fringes of the droplet. The particles can be repeatedly agitated inside the droplet by alternating the polarity of current, as presented in Supplementary Video S2.

In this work, particle agitation was performed by applying a current of 1.5 A with alternating frequency of 0.5 Hz. Performance was evaluated by comparing the diffusion process of the dark blue food dye in water in presence and absence of agitation. Inner convective flow was introduced with the assistance of agitation and accelerated the mixing process, as shown in Fig. 3B. Mixing of food dye with water was achieved in 2 seconds. Conversely, hardly any mixing was observed over the span of 50 seconds when the process was left to diffusion alone, as shown in Fig. 3C. In contrast to the permanent magnet-based actuation scheme, in which the magnetic particles are anchored to the surface due to magnetic attraction or higher specific gravity, mixing efficiency can be enhanced by means of magnetic agitation in the proposed system.

3.5 Particle washing

When crude biological samples are lysed, the buffer carried over by the magnetic clusters contains PCR inhibitors including hemoglobin and DNA-binding proteins (Wilson, 1997). Performance of washing process was evaluated by estimating the volume of buffers being carried over between each reagent droplet. Washing process was defined as a combination of droplet splitting with subsequent merging. Owing to the presence of a dark blue dye in lysis/binding buffer, it is a suitable indicator of cleanness after each washing step. A reference curve was first generated by measuring the mean gray value of color of lysis/binding buffer against serial dilutions to evaluate the effect of washing (Supplementary Fig. 3). Afterwards, the magnetic particles were split from LSB droplet and were subsequently washed in WB1a, WB1b, WB2a, and WB2b, as demonstrated in Fig. 4A. The washing sequence is also presented in Supplementary Video S3. Fig. 4B presents the mapped grey values of LSB, WB1a, WB1b, and WB2a on the reference curve with dye concentrations of 83.9% (C_{LSB}), 6.1% (C_{WB1a}), 0.5% (C_{WB1b}), and less than 0.1% (C_{WB2a}), respectively.

Based on a previous investigation by Lehmann et al., a simple dilution calculation was performed using the equation $V_C \times C_{LSB} = C_{WB1a} \times (V_C + V_{\text{droplet}})$. The carryover volume (V_C) after the first wash step was estimated to be around 1.2 μL that is merged with a subsequent droplet of a volume (V_{droplet}) of 15 μL . Similarly, the carryover volume after the second washing was estimated to be around 1.3 μL . Volume occupied by the magnetic particles (< 0.1 μL) was considered to be negligible. It is reasonable to estimate that each washing on the device step results in >10 fold dilution, meaning that the lysis buffer residue and PCR inhibitors carried alongside magnetic particles are attenuated 10^4 -fold or greater in the PCR reaction buffer after the four washing steps.

3.6 DNA extraction and on-chip real-time PCR

In order to test the feasibility of applying our fluidic platform to PCR-based diagnostic assays, we first characterized DNA extraction from mammalian cells. Human adenocarcinoma cell line culture (Panc-1) was suspended in phosphate buffered saline and concentration was measured by calculating the average of five aliquots on a hemocytometer

slide. The cells were serially diluted over three orders of magnitude ($10^4 \sim 10^2$ cells per 10 μL aliquot), and processed in triplicates of 10 μL aliquots using the electromagnetic coil platform. Elution buffer from the DNA extraction kit replaced PCR reagents in this experiment. The amount of eluted DNA was quantified using a standard PicoGreen assay (Quant-iT, Invitrogen). In general, extraction yield on the order of 10~20 pg genomic DNA per 10^2 cells was observed (data not shown). Higher yields may be possible by further optimizing cell lysis and elution conditions in the future.

The effect of bead agitation on DNA capture and elution process was also examined. Compared to a diffusion-dependent process, particle agitation would increase the effective volume sampled by the surface of magnetic particles during an incubation process. In order to evaluate this, DNA extraction was performed while omitting agitation in one of the two incubation steps (Supplementary Fig. 4a). Three test cases were performed for comparison, including A) no agitation in elution droplet, B) no agitation in LSB droplet, and C) agitation in both LSB and elution droplets. The effect of agitation on DNA elution was assessed by comparing extracted DNA from cases A and C, while a comparison between B and C would examine its effect on DNA capture. After quantification of eluted DNA using PicoGreen assay, it was observed that bead agitation in LSB enhanced the capture of DNA, as evidenced by the substantial enhancement in the amount of extracted DNA (22.5 folds of enhancement from 0.24 ng to 5.4 ng). Similarly, DNA elution was enhanced by approximately twofold as a result of bead agitation during the elution process. Additional information is presented in Supplementary Fig. 4.

As a proof of concept, real-time amplification of the KRAS oncogene was performed on the droplet platform using 5 μL of human whole blood as the biological sample input. Genomic DNA was first isolated from whole blood using automated processing described in an earlier section and eluted directly into a PCR reagent mixture droplet containing $1 \times \text{LCGreen+}$. The processed cartridge was subsequently mounted on a custom thermal cycling and detection module and amplified while monitoring the fluorescence emission of the droplet.

Fig. 5A shows the real-time PCR signal from KRAS detected within the droplet. We observed a high threshold cycle number that was attributed to lower amplification efficiency, associated with long transition times between temperature zones and the effect of thermal gradient across the droplet. Optimization of thermal cycling may be achieved by modification of the cartridge design to reduce and isolate the thermal mass associated with the PCR mixture chamber, while modifying the heating unit to increase contact area between heating surface and the PCR chamber. In order to verify that the amplification signals were associated with the target region rather than primer dimers or unspecific products, the amplified product was analyzed using 2% agarose gel electrophoresis. Positive control samples were generated by thermal cycling PCR mixtures spiked with 2 ng male genomic DNA (Promega Corporation, USA) under similar cycling conditions on a conventional thermal cycler, while negative control was generated by thermal cycling the same mixture without genomic targets (no template control). As shown in Fig. 5B, gel results show that the products were of expected length (Lane 1), which confirmed amplification of target product from the sample.

4. Conclusions

This study demonstrates an automated magnetic droplet-based system for whole blood genetic testing, capable of nucleic acid extraction from crude samples, nucleic acid amplification, and real-time fluorescence detection on a single disposable cartridge. Topographical barriers on the cartridge were used in tandem with magnetic coil-based instrument to create a simple and efficient droplet manipulation scheme. Planar coil

structures provided a ubiquitous actuation mechanism for the splitting, transport and agitation/mixing of magnetic particles, while topographical barriers enabled efficient splitting and isolation of reagent droplets with varying interfacial tensions. Using this platform, automated genomic DNA extraction from whole blood was achieved in 15 minutes, which is a fraction of about 1 hour in a laboratory bench-top process.

The cartridge was interfaced with external thermal cycling and optical detection modules to successfully demonstrate real-time amplification detection of genetic target. A major strength of the proposed droplet manipulation platform is its flexibility, as the platform can simultaneously handle a diverse range of reagents and could be extended to perform other assays using magnetic particles as a substrate for biomolecule isolation and detection. We envision that the developed platform could deliver nucleic-acid based diagnostic assays to a point-of-care setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- An electromagnetically actuated fluidic platform for decentralized genetic testing is presented.
- The operating mechanism utilizes solid phase extraction of nucleic acids with silica-coated magnetic particles for integrated sample processing and detection.
- Combination of topographical barriers and electromagnetic actuation enables robust fluidic handling capabilities, including agitation/mixing and particle splitting in a wide range of reagents.
- Automated DNA extraction is performed using the proposed platform in 15 minutes.
- Extraction of human genomic DNA from unspun whole blood and subsequent amplification using real-time PCR is performed on a single cartridge.

Fig. 1.

Droplet manipulation platform. (A) Topography-assisted droplet splitting. Magnetic particle cluster was extruded from the parent droplet as the magnetic attraction moved the cluster through a sieve structure. A wide-range field gradient was generated by creating magnetic fields of opposing polarity on two adjacent coils. (B) Splitting is achieved by applying a strong field gradient between two droplet compartments. Negative, zero and positive polarity currents are applied in 3 consecutive coils to generate field maximum at the center of destination droplet and field minimum at the center of current droplet. The resulting force applied on magnetic particles facilitates droplet splitting. (C) Agitation is achieved by alternating between two polarities of current applied to a single coil directly below the droplet containing magnetic particles. When the field is directed upward, gradient maximum is located at the center of the droplet and particles are concentrated. In reverse polarity, gradient is directed away from the center of the droplet and force is applied on particles to move towards the fringes of the droplet.

Fig. 2.

Photographic sequences showing droplet splitting and merging assisted by a sieve structure. From left to right: (A) magnetic particles were dispensed into the parent droplet containing yellow food dye, forming columns due to magnetization under large transverse B-field; (B) magnetic particles collected towards the maximum of coil-generated field gradient, located at the center of the sieve structure; (C) field gradient maximum was changed to the center of the adjacent droplet, causing magnetic particles to pull through the sieve structure; (D) this process formed a long neck, which reduces capillary force until a sessile plug containing magnetic particles was formed; (E-F) the separated plug was magnetically transported and merged with the adjacent droplet.

Fig. 3. Photographic sequences showing droplet agitation. (A) Left: magnetic particles attracted towards the center of the droplet. Center: particles dispersed towards the fringes of the droplet. Right: particles dispersed inside the droplet when no current is applied to the coil. (B) Mixing of blue food dye in water droplet with the assistance of agitation. (C) Diffusion of blue food dye in water droplet in absence of agitation.

Fig. 4.

On-chip droplet manipulation for sample washing. All images were converted to gray scale for analysis. (a) Photographs of sequential splitting, merging, and washing performed in an automated fashion. The arrows indicate magnetic particle clusters. (b) Potential carryover of PCR inhibitors can be minimized by successive washing steps. Droplet manipulation in the demonstration above was performed with 300 μg particle load and 15 μL reagent droplets each.

Fig. 5. Amplification signals obtained from PCR on chip. (a) Real time PCR amplification curve. Amplification curve (black solid line) is generated by smoothing raw signal () with 8th order Savitsky-Golay filter. (b) Gel electrophoresis image of PCR products. The amplified products (Lane 1) were verified by comparing against positive controls (Lane 2) and no-template controls (Lane 3) amplified from male genomic DNA on a conventional thermal cycler using 2% agarose gel electrophoresis. Products are 167bp in length.

Scheme 1.

(A) Photograph of the disposable chip containing aqueous food dyes pre-injected in each compartment. (B) Schematic illustration of magnetic droplet manipulation system composed a disposable chip and actuation module for automated nucleic acid extraction. (C) Diagram illustrating alignment of droplet cartridge on coil PCB. Top layer of coils were centered at each droplet compartments (yellow, solid lines), which would automatically align the bottom layer of coils (black, dashed lines) to the centers of sieve structures. (D) Layout of droplet cartridge and sample processing order indicated by black arrows. Droplet cartridge was composed of seven compartments separated by sieve structures to facilitate droplet splitting. Each compartment was primed with reagent droplets and overlaid in mineral oil. The magnetic particles were split from the lysis/binding buffer droplet and transferred to washing buffers WB1a, WB1b, WB2a, and WB2b for DNA extraction. Afterwards, the particle-bound DNA was directly eluted in PCR reagent droplet, followed by extraction of particles into the last compartment.