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Effect of surfactants on carryover liquid volume in immiscible phase magnetic bead separation

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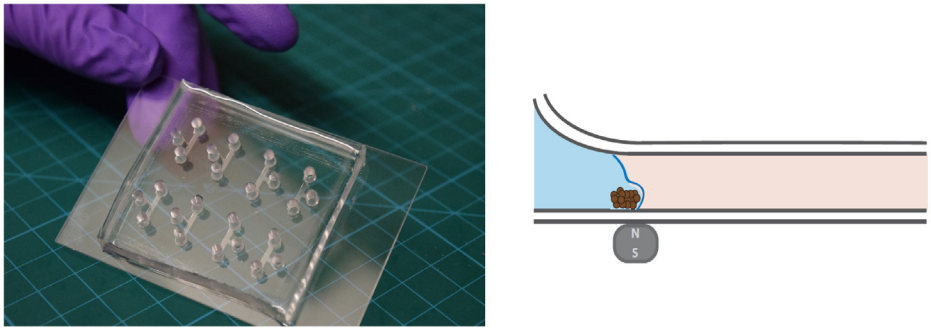
HIGHLIGHTS

- Carryover liquid is characterized at different interfacial tensions.
- Bead aggregate formation is function of interfacial tension.
- Carryover volume is function of bead aggregate geometry and bead mass.

GRAPHICAL ABSTRACT



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ABSTRACT

This manuscript describes the transport of beads across an interface of two immiscible liquids, an aqueous phase and an oil phase, through a microfluidic channel and across another interface into an elution reservoir. This technique can be used for isolating molecules that bind to the beads from the surrounding aqueous material, i.e separation of particular biomolecules from a sample. However, the interface allows a small volume of aqueous liquid to be carried over along with the beads. Carryover liquid from sample to elution reservoir may be detrimental in downstream applications like polymerase chain reactions. Here we quantify carryover volume in relation to different surfactant and protein doped sample liquids. Our data suggest that the carryover volume increases rapidly with an increase in the oil-liquid interfacial tension (γ) up to a critical interfacial tension, $\gamma > \sim 20$ dynes/cm, at which point the carryover volumes remain roughly constant. Our experiments also show that lower values of γ produce more numerous smaller bead clusters while higher γ allows movement of fewer large bead clusters. Lastly, we identify two modes of liquid carryover, interstitial and enveloping, and the conditions under which either occur. We discuss our results in comparison to literature studies.

1. Introduction

Sample preparation is an important step for many diagnostic tests, especially ones involving nucleic acid amplification. In order to amplify nucleic acids, it is imperative to begin with a clean, PCR-inhibitor free sample. Traditionally, the process of isolating nucleic acids from a large, complex sample has been cumbersome, time consuming and costly in materials [1–4]. Along with the requirement of laboratories with expensive equipment and trained personnel, the limited availability of effective and inexpensive sample preparation makes nucleic acid testing in low resource settings, where infectious diseases are most prevalent and where diagnostic

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tests are most needed, difficult to implement [1–3]. In general, there are four steps to nucleic acid extraction and purification: cell/tissue disruption, nucleoprotein denaturation, inactivation of nucleases and separation from cell/tissue debris [5]. Bloom et al. was able to contribute to nucleic acid isolation with the introduction of solid phase extraction utilizing glass particles, which has evolved into the use of magnetic or paramagnetic particles [6]. In this technique, magnetic particles with an iron oxide core use a cellulose-based surface chemistry to bind exposed nucleic acids. Several wash steps are performed to remove cell and tissue debris, as well as residual lysis reagents which could inhibit downstream applications, while a magnet is used to hold the nucleic acid bound magnetic particles stationary within a tube [5]. Finally, an elution buffer is added to release the nucleic acids from the magnetic particles into the supernatant. Although this method can produce adequate nucleic acid yields, it is time and labor intensive, and has the potential for high end user error which may result in lower yields. Most of the commercially available extraction methods follow the above workflow.

Current research aims to consolidate and improve the process of magnetic bead separation [3,7–11]. This is being achieved through the use of low cost polymer materials and through the use of microfluidics, the latter of which offers the advantages of having very fast reaction times and large surface to volume ratios [9,12]. Many systems have been developed to move paramagnetic particles from a lysis well to an elution well, eliminating the need for multiple wash steps and thus reducing the number of reagents required [7]. These systems mix magnetic particles in an aqueous phase containing free nucleic acids and allow for adsorption of the nucleic acids to the surface of the particles. Then a magnet is used to drag the nucleic acid bound beads through a second phase that is immiscible with the first, and into an elution buffer [7,8,13–22]. The magnetic force pulls the beads bound with nucleic acid through the system while the surface tension between the two phases prevents the rest of the sample from following. Recently, we have shown that 1 μg of beads provides a large enough surface area for the capture of nucleic acids present in patient samples[23]. However, most previous studies were performed using high bead loads (10–5000 μg) which provide high magnetic forces for bead clusters to penetrate through oil-liquid interfaces (see Table 1). Moreover, multiform mechanisms or no mechanisms have been heretofore offered for the observed carryover volumes. More experimental studies are needed to explore the fundamental mechanisms and insights into bead transfer through oil-liquid interfaces. Here we explore bead transfer through oil-liquid interface using a small mass of beads which provide sufficient surface area for capturing target molecules [24]. The aim of this paper is to both quantify carryover volume and study the relationship between it and interfacial tension.

2. Materials and methods

2.1. Materials

Olive oil (Fillippo Berio Extra Light Tasing Olive Oil), nuclease free water (Integrated DNA Technologies), human plasma (Sigma-Aldrich), 5 M Saline (Ambion), TE Buffer (Integrated DNA Technologies) and MagMax Viral RNA isolation lysis buffer (Ambion) were each purchased and used as a part of control measurements. Out of the many commercially available paramagnetic particles ([25]), ambion RNA Binding Beads (AM1939, F10019G11) are used. SDS (Sigma-Alderich) Tween-20 (Sigma) and BSA (New England Bio Labs) were purchased and used as variable conditions for our experiments.

2.2. Chip fabrication

The Polydimethylsiloxane (PDMS) chip used for extraction experiments consists of three wells, one long channel (6 mm) and one short channel (1 mm) connected in a sideways "T" shape as shown in Fig. 1. Both channels are 1.25 mm wide and 150 μm deep. The wells are all 3 mm in diameter and 5 mm in depth. A PDMS microchannel chip was fabricated over a SU-8 substrate on a silicon wafer as the mold. Channels were formed by setting the PDMS precursor mixed with curing agent (Sylgard 184 Corning Coperation) into the mold and allowing for polymerization in a 70 °C incubator for approximately 1 h. The solid PDMS gel is then cut out of the mold and three holes are punched into the ends of each channel to form wells. After treating the glass slide and PDMS with plasma, the gel is bonded to a glass slide, creating a channel with 3 PDMS sides and one glass side.

2.3. Priming the chip

10 μL of oil is pipetted into the middle well (well 3, Fig. 1b) and allowed to fill the channel. 10 μL of priming buffer, 1 mg/mL BSA and 0.01% Tween-20 in nuclease free water, is then quickly pipetted into the sample and elution wells. The primed chip is then placed in a refrigerator (4 °C) and incubated for at least 10 h before use. In the event that the interface was visibly distant from the sample well (i.e. located more than 1 mm into the oil channel), the device was discarded and not used for these experiments.

2.4. Separation

In a conical tube, 2 μL of beads are mixed with 107.8 μL of surfactant or BSA at varying concentrations in either nuclease free water or plasma along with 12.2 μL of 10 μM fluorescein. The number of beads was kept constant for all experiments. After the sample is vortexed, the beads are concentrated at the bottom of the conical tube using a neodymium magnet of ½" diameter and 3/8" thickness (K&J Magnetics, Inc. D86-N52). To begin the extraction experiment, the primer is removed from well one. Then, capturing as many of the beads as possible, 10 μL of solution is pipetted out of the tube and into well 1. Primer is then removed from well 2 and replaced with 10 μL of TE buffer (to simulate the eluent). A magnet is placed below the glass slide at the threshold of well 1 and the oil channel. Accounting for the 1 cm glass cover slip between the magnet and the beads/sample, we estimate, using manufacturer specifications, the magnetic field strength acting on the beads to be around 4000 G. The beads are given time to form an aggregate and then, once formed, the magnet slowly traces the path of the oil filled channel. The beads are moved at a speed of approximately 1 mm/s. Previous studies in our microchip system report this as the optimal speed for bead movement [24]. Higher speeds can cause a loss of aggregate momentum. Care is given to bring as much bead aggregate as possible into well 2. The sample and beads are pipetted out of the well and the supernatant is separated from the beads using a magnet. The supernatant is then used as the sample for quantification of fluorescein concentration. All of the samples were analyzed simultaneously with a fluorescein calibration curve in a plate reading fluorometer (BMG Labtech PHERAstar). Using the calibration curve, the concentration of fluorescein in well 2 was determined, from which the volume of liquid carried over from well 1 could be quantified. Olive oil and nuclease free H_2O were tested for background fluorescence, which was found to be negligible. We recorded an average of 110 RFU for olive oil while the calibration curve from 0.05 μM to 1.5 μM amplified between 1886–67345 RFU. Our nuclease free H20 blank had an average of 13 RFU.