CONTINUOUS-FLOW AND LABEL-FREE FERROHYDRODYNAMIC SORTING OF MAMMALIAN CELLS IN BIOCOMPATIBLE FERROFLUIDS
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ABSTRACT
This paper reports a new continuous-flow and label-free sorting scheme based on biocompatible ferrofluid hydrodynamics (ferrohydrodynamics) for the separation of mammalian cells (HeLa cells and mouse whole blood). Ferrofluids are stable magnetic nanoparticles suspensions used as sorting media in microfluidics for both particles and cells. Here we develop a biocompatible ferrofluid that can sustain the viability of mammalian cells for up to several hours with excellent colloidal stability and tunable concentration to allow for cell observation without fluorescent labels. We apply this ferrofluid in continuous-flow sorting of HeLa cells and blood cells with high throughput and high separation efficiency.

KEYWORDS: Biocompatible, Label-Free, Cell Sorting, Ferrofluid.

INTRODUCTION
Microfluidic magnetophoresis has been used to separate cells and beads with different magnetic susceptibilities [1]. It requires the magnetic labeling of cells to facilitate the magnetic force-based manipulation. To avoid this time-consuming and manually intensive step, “negative magnetophoresis” has been developed for cell manipulation. Non-magnetic cells placed inside a paramagnetic salt solution or ferrofluid [2] medium are pushed away when exposed to an external magnetic gradient field. Ferrofluids have relatively high magnetic susceptibility compared to paramagnetic solutions. However, attempts to use water-based ferrofluids for mammalian cell manipulation have been hampered by cell visibility and biocompatibility issues. In this study, a customized ferrofluid with pH 7, balanced salt concentration, and biocompatible polymer coated maghemite nanoparticle is developed for separation of HeLa cells and blood cells. It maintains the viability of HeLa cells and blood cells and provides good visibility in microfluidic channels without any labeling. The long term goal of this project is to apply this method to cervical cytology specimens to separate rare abnormal cells from inflammation, blood and normal cells.

The separation device consisted of a microchannel and a permanent magnet (Fig. 1(a) and (b)). When the magnet was not present near the microchannel, cell mixture and ferrofluids were injected into the channel by a pressure-driven flow. Fig. 1(a) shows larger HeLa cells and smaller blood cells entered and exited the microchannel together, resulting in no separation. However, when the magnet was near the microchannel, deflections of cells from their laminar flow paths occurred because of the magnetic field gradient and resulting magnetic buoyancy force [3]. This deflection will lead to spatial separation of cells of different sizes at the end of channel (Fig. 1(b)).

EXPERIMENTAL
The microfluidic device was fabricated through a standard soft-lithography approach with two polydimethylsiloxane (PDMS) layers bonded together (Fig. 1(c) and (e)). Dimensions of the microfluidic channel are listed in Fig. 1(d) and (f). A removable NdFeB permanent magnet was embedded into PDMS 1 mm away from the channel with the magnetization direction perpendicular to the channel. To develop the ferrofluids, maghemite nanoparticles were synthesized by chemical co-precipitation then mixed with polyethylene glycol (PEG). The resulting ferrofluids were dialyzed against water for one week. Excess water was vaporized in a convection oven at 90 °C. Ten-times concentrated Hank’s Balanced Salt Solution (HBSS) was added into the ferrofluids with a volume ratio of 1:9 to render the liquid isotonic to mammalian cells (HeLa cells and mouse whole blood) in a continuous concentration to allow for cell observation without fluorescent labels. The resulting ferrofluids were dialyzed against water for one week.
mammalian cells. Viability of HeLa and mouse blood cells were tested before the sorting experiments. Nominally $2 \times 10^6$ cells were centrifuged twice at $4^\circ$C and washed in HBSS. For either cell type in duplicate, the washed cell pellet from centrifugation was combined with 1 mL of either 0.4%, 0.79% or 1.03% (v/v) maghemite ferrofluids. After 2 hours of incubation at room temperature in these fluids, cell viability was determined with Trypan blue exclusion and counted with a hemocytometer. For sorting, cells were introduced into Inlet A (Fig. 1(c)) at a constant flow rate of $8 \mu$L/min, and hydrodynamically focused by a sheath flow from Inlet B at a flow rate of $14 \mu$L/min.

Fig. 1: (a) Schematic representation of continuous-flow, label-free mammalian cells sorting in biocompatible ferrofluids. Cell mixture enters and exits the channel together when magnetic field is not present. (b) Larger cancer cells are deflected more than smaller blood cells vertically towards upper outlets when magnetic field is present. This results in a spatial separation of cell mixture. (c) Schematics of the sorting device with the permanent magnet and a microfluidic channel. (d) Top-view of the device and relevant dimensions. Red arrow indicates direction of magnet’s magnetization. Red dashed box indicates the location of the observation window in Fig. 2(b)-(d). (e) An image of prototype device. Scale bar is 10 mm. (f) Cross-section of the device.

RESULTS AND DISCUSSION
The customized 0.4%, 0.79% and 1.03% maghemite ferrofluids were not detrimental to the viability of blood cells or HeLa cells even after 2 hours of exposure. This allows ample time to carry out the sorting experiments (Fig. 2(a)). When the magnetic field was off, all cells were flowing near the side wall of the channel and exiting through Outlets 1 and 2 (Fig. 2(b)). When magnetic field was present, magnetic buoyancy force deflected HeLa cells from their laminar flow paths towards Outlets 5 and 6 (Fig. 2(c)). Meanwhile, force on mouse blood cells was insufficient to deflect them above Outlet 4, resulting in spatial separation of cell mixture at the end of channel. After separation, cell numbers at each outlet were counted with hemocytometer and analyzed for cell distributions. Sample images from Outlet 1 (Fig. 2(d)) and Outlet 6 (Fig. 2(c)) were taken for better visualization with the help of cytospin and Wrights-Giemsa staining. From the size distribution analysis as in Fig. 2(e), 97.1% HeLa cells were collected in Outlet 6, the remaining smaller HeLa cells were collected in Outlet 5.

CONCLUSION
We have developed a biocompatible ferrofluid that can sustain the viability of mammalian cells for up to several hours with excellent colloidal stability and tunable concentration to allow for cell observation without labels. We apply this ferrofluid in the continuous-flow sorting of HeLa cells and mouse blood cells with high throughput and high separation efficiency. Tuning the device to separate other cells and testing on human cancer cytology specimens could be further advanced in our future work.
Fig. 2: Experimental results of mammalian cell sorting. (a) Viability of mouse whole blood and HeLa cells in PEG surfactant stabilized ferrofluids as function of time. (b-d) Left, Refractive index of cell chamber and cells at outlet (outlet = red number, scale bar = 300 µm). Right, Wright-Giemsa stain of cytospin of cells exiting outlet (scale bar = 50 µm for b and c, 20 µm for d). (b) In absence of magnetic field, cell mixture exits Outlets 1 and 2. Cytospin shows mixture of large HeLa cells (light purple nucleus and pale blue cytoplasm) and red blood cells. The deep purple cells, smaller than standard HeLa cells, are condensed HeLa cells, and possibly white blood cells. (c) and (d) When magnetic field is applied, HeLa cells exit in Outlets 5 and 6 and blood cells exit in Outlets 1, 2, and 3. (e) Counting of cell types and numbers at each outlet.

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REFERENCES

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