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Graphical and textual abstract

We proposed and developed a novel viscoelastic ferrofluid, and demonstrated its superior advantages for continuous sheathless separation of non-magnetic particles.

(a) Schematic illustration of microparticle viscoelastic 3D centreline focusing and magnetophoretic separation in the PEO-based ferrofluid.
(b) Numerical simulation and experimental validation of viscoelastic focusing and magnetophoretic separation.
A novel viscoelastic-based ferrofluid for continuous sheath-less microfluidic separation of nonmagnetic microparticles

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Abstract:
Separation of microparticles has found broad applications in biomedicine, industry and clinical diagnosis. In a conventional aqueous ferrofluid, separation of microparticles usually employs a sheath flow or two offset magnets to confine particle streams for downstream particle sorting. This complicates the fluid control, device fabrication, and dilutes particle sample. In this work, we propose and develop a novel viscoelastic ferrofluid by replacing the Newtonian base medium of the conventional ferrofluid with non-Newtonian poly(ethylene oxide) (PEO) aqueous solution. The properties of both viscoelastic 3D focusing and negative magnetophoresis of the viscoelastic ferrofluid were verified and investigated. By employing the both properties in a serial manner, continuous and sheathless separation of non-magnetic particles based on particle size has been demonstrated. This novel viscoelastic ferrofluid is expected to bring more flexibility and versatility to the design and functionality in microfluidic devices.

Introduction
Manipulation of microparticles in a microfluidic platform is an indispensable tool for biomedical applications. For example, three-dimensional (3D) focusing and ordering of micro-particles along a specific single path enables single-cell level detection and analysis in on-chip flow cytometry. Focusing randomly distributed particles into one or several positions can be employed to enrich \textsuperscript{1} or filtrate \textsuperscript{2} bioparticles. Separation of target bioparticles from a massive background of bioparticles according to the unique biophysical properties is a routine process in medical laboratories for downstream biochemical analysis, disease diagnosis and therapeutics \textsuperscript{3}. 
Many techniques have been developed to manipulate microparticles in microfluidics. Depending on the source of the manipulating forces, they are categorised as active and passive techniques. Active techniques rely on external electric, magnetic, acoustic or optical force fields, whereas passive techniques depend entirely on the channel geometry and intrinsic hydrodynamic forces, such as pinched flow fractionation, deterministic lateral displacement, hydrophoresis, inertial microfluidics and viscoelastic focusing.

Magnetophoresis was first proposed to describe the behaviour of a magnetic particle moving through a viscous medium under the influence of an external magnetic field. The functionalized magnetic beads were used to label bioparticles because most of bioparticles in nature are non-magnetic. However, the label-based methods are labour-intensive and time-consuming, and the magnetic moments of beads may vary significantly. In order to circumvent the problem, a label-free technique that uses negative magnetophoresis to manipulate and separate non-magnetic bioparticles was proposed by suspending them in a magnetic fluid, such as paramagnetic salts or ferrofluid. In negative magnetophoresis, effective magnetic dipole moments are induced within the microparticles, which experience a magnetic buoyancy force along the weaker field direction under non-uniform magnetic fields. Paramagnetic solutions such as MnCl₂ and GdCl₃ have a poor magnetic susceptibility. In order to induce sufficient magnetophoresis of particles, salt concentration must be sufficiently high which may destruct its biocompatibility. Alternatively, strong magnets are required to be brought very close to the nonmagnetic particles.

Ferrofluid is an opaque colloidal suspension of magnetic nanoparticles (made of magnetite, Fe₃O₄, and usually of 10 nm in diameter) in pure water or organic solvent with surfactants coated to prevent cohesion. Synthesis of biocompatible ferrofluids has been reported using appropriate stabilizing surfactants. Because ferrofluids usually have a much higher magnetic susceptibility than that of paramagnetic solutions, permanent magnets or electromagnets are sufficient to induce enough magnetophoretic force to manipulate non-magnetic particles in microfluidics. Many previous studies have reported focusing and separation of microparticles and cells using ferrofluid in microfluidics. In those studies, the non-magnetic particle suspension needs to be first confined by a co-flowing sheath flow; and in the downstream, an external magnetic field acts on the nonmagnetic particles and deflects them into different paths. However, the utilization of sheath flow not only complicates the flow control and device fabrication, but also dilutes the sorted particles. Alternatively, the employment of two offset magnets or two arrays of permanent magnets in a straight microchannel has been proposed to achieve sheath-free
focusing and continuous separation of particles. The main challenge of using two offset magnets lies in positioning two magnets precisely in the microfluidic device because there is a strong magnetic attractive or repulsive force between two magnets. Furthermore, determining the exact distances between two offset magnets and microchannel is laborious.

In conventional ferrofluids, Newtonian fluid (an organic solvent or water) is usually used as the carrier medium. Recently, the elastic property of non-Newtonian fluid has been extensively studied to manipulate microparticles in microfluidics \(^{27, 28}\). The proposed viscoelastic mediums for particle viscoelastic focusing include solutions of poly(ethylene oxide) (PEO) \(^{29-32}\), polyvinylpyrrolidone (PVP) \(^{3, 33}\), DNA \(^{34}\), polyacrylamide (PAM) \(^{35}\) and hyaluronic acid (HA) \(^{36}\). The elastic force induced by the normal stress difference in these viscoelastic fluids will focus particles into the zero shear rate regions (channel centerline or corners in a square channel) \(^{34, 37}\). Under a proper flow rate, the multiple equilibrium positions can be further reduced to one at the centreline due to the synergetic effects of inertial and viscoelasticity \(^{38}\).

In this work, we explore the possibilities of combining these two properties (negative magnetophoresis and viscoelastic focusing) in a single medium, by suspending magnetic nanoparticles in a viscoelastic carrier medium. The individual properties of negative magnetophoretic and viscoelastic focusing will be verified and investigated. Then, through combining both properties in the design of microfluidic devices, sheath-less size-dependent separation of non-magnetic microparticles will be demonstrated through viscoelastic pre-focusing and magnetophoretic deflection in a serial manner. Empowering both superior properties in a single ferrofluid is expected to bring more flexibility and versatility for the design and implementation of particle focusing and separation in microfluidics.

2. Mechanism

2.1 Negative magnetophoretic force

When non-magnetic particles suspended in a ferrofluid are exposed to a non-uniform magnetic field, the negative magnetophoretic force on the non-magnetic particles is expressed as \(^{15, 17}\):

\[
F_m = 3\mu_0 V_p \frac{\chi_p - \chi_f}{3 + \chi_p + \chi_f} (H \cdot \nabla)H
\]  

(1)

where \(\mu_0 = 4\pi \times 10^{-7}\) H/m is the permeability of free space, \(V_p\) is the volume of the particle, \(\chi_p\) is the magnetic susceptibility of the particle, \(\chi_f\) is the magnetic susceptibility of the ferrofluid, and \(H\) is the magnetic field at the centre of the particle. Because the magnetic susceptibility
of the non-magnetic particle is smaller than that of ferrofluid, \(\chi_p < \chi_f\), the negative magnetophoretic force \(F_m\) directs along the inverse direction of magnetic field gradient. When \(\chi_p\) and \(\chi_f\) are at least three orders of magnitude smaller than 1 for the nonmagnetic particles and the diluted ferrofluid, equation (1) can be further simplified as 6:

\[ F_m = \mu_0 V_p(\chi_p - \chi_f)(H \cdot \nabla)H \]  

(2)

### 2.2 Elastic force

Particles immersed in a viscoelastic fluid experience an elastic force due to the intrinsic properties of the suspending medium. The elastic effects of a viscoelastic fluid in channel flow can be characterised by the Weissenberg number \(W_i\):

\[ W_i = \frac{\lambda}{\tau_f} = \lambda \dot{\gamma} \]  

(3)

where \(\lambda\), \(\tau_f\) and \(\dot{\gamma}\) are the relaxation time of the viscoelastic fluid, the characteristic time of the channel flow and the average fluid shear rate, respectively. The characteristic time is approximately equal to the inverse of the characteristic shear rate \(\dot{\gamma} (= 2Q/hw^2)\), which is defined as the ratio of the average flow velocity \(U = Q/hw\) to the half channel width \(w/2\) 29, 31, 39. Here \(Q\) is the flow rate, \(w\) and \(h\) are the width and height of the microchannel, respectively. In a viscoelastic fluid, both the first and second normal stresses, \(N_1 = \tau_{xx} - \tau_{yy}\) and \(N_2 = \tau_{yy} - \tau_{zz}\) contribute to particle lateral migration; here \(\tau\) is the normal stresses that are exerted in the flow. Because \(N_1\) is much larger than \(N_2\) in the diluted PEO solutions, the effects of \(N_2\) can be neglected 40, 41. The elastic force \(F_E\) originates from an imbalance in the distribution of \(N_1 = \tau_{xx} - \tau_{yy}\) over the dimension of the particle 29, 38, 42.

\[ F_E \sim a^3 \nabla N_1 \sim a^3 W_i \dot{\gamma}^2 \]  

(4)

where \(a\) is the particle diameter.

### 2.3 Stokes drag

Drag force arises when an object moves in a different velocity with the corresponding fluid elements. The origin of the drag force lies in the need to displace the elements of the fluid out of the way of the moving object. The drag force on a moving spherical particle can be expressed by Stokes law:

\[ F_d = 3\pi \mu a (u_f - u_p) \]  

(5)

where \(\mu\) is the dynamic viscosity of fluid, \(u_f\) and \(u_p\) are the velocity vectors of fluid and particles respectively.

### 2.4 Particle dynamics
Three forces act on particles suspended in a viscoelastic ferrofluid, and the motion of particles is determined by the sum of these forces:

\[ m_p \ddot{X} = F_m + F_E + F_d \]  

(6)

where \( m_p \) and \( \ddot{X} \) are the mass and acceleration of particles. It should be noted that particles also experience other forces, such as gravity and buoyancy force, and they are negligible because the density of particles and the suspending medium are very close. In our design, the dominance of each force is implemented at different regions of the microchannel for the proper functionalities. In a straight channel with a square cross-section, the elastic force \( F_E \) dominates the motion of particles, so that particles can be focused along the channel centreline. Then in the downstream, differential lateral migration of non-magnetic microparticles is controlled by the magnetophoretic force \( F_m \) to enable size-based separation. At the end of the microchannel, separated particles follow the fluid streamline by the dominant Stokes drag \( F_d \) and enter the corresponding outlets.

3. Materials and methods

3.1 Design and fabrication of microfluidic device

In this study, the microchannel in the microfluidic device consists of three straight channel sections, with dimensions of 50 \( \mu \text{m} \times 20 \text{ mm} \), 600 \( \mu \text{m} \times 10 \text{ mm} \), and 800 \( \mu \text{m} \times 5 \text{ mm} \) (width \( \times \) length), respectively. A linear expansion region between two sections was used to connect them smoothly. A rare earth neodymium (NdFeB) (4mm\times4mm\times4mm) was placed on one side of the second straight section with a lateral distance about 1 mm, Figure 1a. The height of the microchannel is 56 \( \mu \text{m} \), Figure 1b. Microfluidic devices were fabricated by the standard photolithography and soft lithography techniques\(^{43}\). A three-dimensional illustration of the microfluidic device and the image of a fabricated microfluidic device are shown in Figures 1c and 1d, respectively.
Figure 1 (a) Geometry of the microchannel and magnet. (b) Cross sectional view of the microchannel and magnet. (c) 3D illustration of the microfluidic device. (d) Image of the fabricated microfluidic device (the microchannel is filled with blue food dye for clarity).

3.2 Preparation of PEO-based ferrofluid

PEO (poly (ethylene oxide), Mw=2 000 000, Sigma-Aldrich, Australia) aqueous solutions with concentrations of 1000 ppm and 2000 ppm were first prepared by dissolving PEO powder in DI water. Then, mixing the PEO solution (2000 ppm) with a commercial water-based magnetite ferrofluid (EMG 408, Ferrotec Co., Singapore, the volume ratio of the magnetite nanoparticles is 1.1%) and DI water in a ratio of 5:1:4 resulted in a ferrofluid with the PEO solution as the base medium. This novel fluid is called PEO-based ferrofluid. In this mixture, the PEO concentration is 1000 ppm, and the volume ratio of magnetite nanoparticles (mean diameter = 10.2 nm) is 0.11%, which is 1/10 of the original ferrofluid. Meanwhile, the aqueous ferrofluid with the same volume ratio of magnetite nanoparticles of 0.11% was prepared by diluting the commercial ferrofluid with DI water by 10 times.

3.3 Particle preparation

Fluorescent polystyrene particles with a mean diameter of $a = 5 \, \mu m$ (Product No. G0500, CV5%) and 13 $\mu m$ (Product No. 36–4, CV16%) were purchased from Thermo Fisher Scientific. In the verification tests, 5-$\mu m$ particles were suspended in the proposed PEO-based ferrofluid, to confirm both features of viscoelastic focusing and negative magnetophoresis in the proposed PEO-based ferrofluid. To conduct the separation tests,
binary polystyrene beads mixture (5 µm and 13 µm) was suspended in the proposed PEO-based ferrofluid, as well as in the conventional aqueous ferrofluid for comparison. The concentrations of polystyrene particles in these suspensions are ~ 10^6 counts/ml. Tween 20 (Product No. P9416, Sigma-Aldrich, Australia) with 0.1% w/v was added to prevent particles from aggregation.

### 3.4 Experimental setup

The NdFeB permanent magnet creates a non-uniform magnetic field. The magnet was placed on one side of the wide microchannel as shown in Figure 1c. The magnetic flux density at the center of the magnet’s pole surface was measured to be 120 mT by a Gauss meter (Model 5180, Pacific Scientific OECO, Cole-Parmer, Australia). The magnetization direction of the magnet is perpendicular to the microchannel. The microfluidic device was placed on an inverted microscope (CKX41, Olympus, Australia), illuminated by a mercury arc lamp. The particle suspension was infused by a syringe pump (Legato 100, KD Scientific, Australia). The fluorescent images were captured by a high-speed CCD camera (Optimos, Q-imaging, Australia), and then post-processed and analysed using the software Q-Capture Pro 7 (Q-imaging, Australia). The exposure time of each frame was 0.1 ms to capture images of single particle. To better observe the trajectories, 100 consecutive frames were merged together.

### 4. Results and Discussion

#### 4.1 Negative magnetophoresis

To confirm the negative magnetophoresis in the PEO-based ferrofluid, 5-µm fluorescent polystyrene beads were dispersed in the proposed medium, and infused into a straight 600-µm wide microchannel. A NdFeB permanent magnet (cube, 4mm in length, height and width) was placed on one side of the microchannel with a distance of 1 mm, and the flow rate was increased from 0.5 µl/min to 15 µl/min through a syringe pump. Figure 2 clearly shows that the non-magnetic particles are repelled from the permanent magnet and migrate toward the opposite side. This proves the existence of the negative magnetophoresis in the proposed PEO-based ferrofluid. In addition, we evaluated the negative magnetophoresis using the average ratio of lateral velocity to horizontal velocity ($V_y/V_x$) of microparticles. Velocity ratio ($V_y/V_x$) decreases sharply when increasing the flow rate from 0.5 µl/min to 5 µl/min, and then much more smoothly from 5 µl/min to 15 µl/min. And when the flow rate reaches 15 µl/min, it is hard to observe the obvious magnetophoretic deflection of particles in the designed microchannel. It should be noted that PEO-based ferrofluid has the property of viscoelasticity, then an elastic force $F_E$ should exert on the micro-particles to push particles along the gradient of fluid shear rate according to equation (4). However, due to the low
aspect ratio (height/width) $\delta \approx 1/12$, the fluid velocity profile along a wide region of channel width is blunted with shear rate constantly zero (Supplementary Figure S1). So the elastic force which is proportional to the gradient of square of shear rate (equation (4)) is zero along a wide central region. Therefore, the influence of elastic force on the magnetophoresis is negligible in this situation.

Figure 2 Negative magnetophoretic deflections of non-magnetic microparticles in the PEO-based ferrofluid under a non-uniform magnetic field. The ratio of lateral velocity to horizontal velocity of microparticles ($V_y/V_x$) decreases sharply with the increment of flow rate when flow rate is ranging from 0.5 µl/min to 5 µl/min, and much more smoothly when flow rate is between 5 µl/min and 15 µl/min. Here, the particle size is 5 µm, microchannel width is 600 µm, and lateral distance between microchannel and the permanent magnet is 1 mm.
4.2 3D viscoelastic focusing

As we know, the viscoelasticity of polymer (PEO or PVP) or DNA solution with long chain molecules can focus micro-particles to the channel centerline or corners in a rectangular channel depending on the initial position of particles due to the gradient of the first normal stress difference. The number of multiple equilibrium positions can be reduced to one at the centreline by increasing the flow rate due to the synergetic effects of inertia and viscoelasticity. To confirm the viscoelastic focusing of microparticles in the proposed PEO-based ferrofluid, 5-µm fluorescent polystyrene beads suspended in the PEO-based ferrofluid were injected into a straight square-shaped microchannel at various flow rates from 5 µl/min to 50 µl/min. As expected, the elastic effects due to the PEO molecule chains in the solution pinches the 5-µm particles gradually into the channel centreline along the channel length, and the existence of magnetite nanoparticles doesn’t alter the viscoelastic focusing, Figure 3a. We also investigated the effects of flow rate on viscoelastic focusing in the PEO-based ferrofluid, Figure 3b. In good agreement with that of pure PEO solution, there is a flow rate threshold (~ 15 µl/min) at which the particle focusing quality is optimal. The particle focusing deteriorates when the flow rate is below or above this threshold. The underlying mechanism of this phenomenon is the competition between the inertia and the elasticity. When the flow rate exceeds the threshold, the inertial lift forces, which usually focus particles at the positions half-way between channel centreline and walls, become much stronger than the elastic force, and deteriorate the centre-directed elastic focusing. However, if the flow rate is too small, inertial lift forces (specifically wall lift force) are too weak to repel particles near four corners towards the centreline, which is also not beneficial for particle focusing at the centreline.
Figure 3 Viscoelastic 3D focusing of microparticles in a microchannel of 50 µm × 56 µm (width × height) (a) along channel length at a flow rate of 10 µl/min, and (b) under different flow rates after 20 mm distance. The particle diameter is 5 µm. The fluorescence intensity profiles are normalized by \( S = \int_0^1 ydx = 1 \).

4.3 Sheathless separation of non-magnetic particles using the PEO-based ferrofluid

After confirming both properties for the proposed PEO-based ferrofluid, a microfluidic device was designed to employ both the features to efficiently separate non-magnetic particles. In order to simplify the design, the two properties of PEO-based ferrofluid were utilized individually in a serial manner. Figure 4a shows that after the introduction of binary micro-particles mixture, a straight microchannel with the square cross section is to focus all the microparticles to the channel centreline due to the elastic force (section I in Figure 4a); Then followed by an expansion region, micro-particles enter a wide straight channel. A permanent magnet placed on one side of microchannel generates a non-uniform magnetic field and exerts a negative magnetophoretic force on the microparticles (section II in Figure 4a). Because the magnitude of magnetophoretic force is highly dependent on particle size, larger particles experience much stronger magnetophoretic force and are repelled more quickly to the opposite wall than the smaller particles (as simulated in Figure 4b). Therefore, a size-dependent separation of non-magnetic particles in the PEO-based ferrofluid can be achieved (supplementary video S1). Here, the roles of a wide channel in section II include: (i) Slowing down the fluid speed from the upper stream, therefore the negative magnetophoretic force can overcome fluid drag force to repel particles toward the minima of the magnetic field; (ii) Low aspect ratio \( h/w\approx1/12 \) creates a blunted velocity profile along channel width as we discussed in the above \( (u = 0 \text{ and } \partial u/\partial y = 0 \text{ for } 0.1w < y < 0.9w, \text{ Supplementary Figure S1}) \), so that the effects of elastic force can be reduced to the minimum in section II. In the following, the 3D pre-focusing by elastic effects and size-dependent deflection by negative magnetophoresis will be demonstrated experimentally.
Figure 4 (a) Schematic illustration of microparticle viscoelastic 3D centreline focusing and magnetophoretic separation in the PEO-based ferrofluid. The viscoelastic effect of the novel ferrofluid is to focus randomly distributed microparticles at the inlet (A-A’) into the channel centreline (B-B’). The expansion region is to decrease the linear fluid flow speed and particle movement speed, so that enough magnetophoretic force can exert on particles for enough time to deflect them. Large particles experience a stronger force and migrate to the opposite wall, while small particles move along the original path due to the weak effects of magnetophoretic force. Therefore, a size-dependent separation of non-magnetic particles can be achieved (C-C’); (b) Numerical modelling of magnetophoretic separation of microparticles by size using COMSOL Multiphysics 5.1. Assuming the successful elastic focusing within the square channel, two differently-sized particles (5-μm and 13-μm) are released from the channel centreline section I, then both particles enter the wide channel (section II) where a non-uniform magnetic field is generated by a permanent magnet. Size-dependent magnetophoretic force exerts on the particles and enables particle separation.
In order to validate the design, a particle mixture was prepared by suspending binary particles of 5-µm and 13-µm diameters in the PEO-based ferrofluid, and then infused into the microchannel at a flow rate of 15 µl/min. The randomly distributed particles in the inlet (Figure 5a) migrate towards the channel centreline due to the effects of elastic force, and both 5-µm (green fluorescence) and 13-µm (red fluorescence) particles focus as a tight streak at the channel centreline after 20 mm (Figures 5b and 5c). After the expansion region, particles still remain as a compact streak even the fluid streamline spreads during the expand region (Figure 5d); When the particles streak encounters the non-uniform magnetic field, larger particles (13-µm) undergo a negative magnetophoretic force 17 times stronger than that of their smaller counterparts (5-µm) because magnetophoretic force is proportional to the volume of particles according to equation (2). Therefore, 13-µm particles are deflected promptly to the opposite side, but the 5-µm particles almost maintain the original position with negligible deflection (Figure 5e). Finally, a bifurcation with three outlets embedded at the end of microchannel collects the corresponding sorted particles (Figure 5f, Supplementary video 2). If the magnet is removed, both types of particles will move straight without any deflection and enter the middle outlet (Figure S3). In addition, we also calculated the separation purity by examining particle mixture before and after separation in the hemocytometer (Figure 5g), and particle purities have been improved significantly from 45.8% to 90.8% for 13-µm particles and from 54.2% to 99.3% for 5-µm particles. This demonstrates the feasibility of the sheath-less separation of non-magnetic particles by employing the both properties of PEO-based ferrofluid in a single microfluidic device.
Figure 5 Sheath-less separation of non-magnetic particles using viscoelastic focusing and negative magnetophoresis using the PEO-based ferrofluid. (a)-(f) are particle trajectories in
Inlet, 10 mm downstream of the straight channel, 20 mm downstream of the straight channel, expansion area, magnet area, and outlet region, respectively. Within these images, (i) bright field images; (ii) and (iii) are fluorescent trajectories of 5-µm and 13-µm particles respectively; and (iv) Composed fluorescent images for the trajectories of 5-µm and 13-µm particles; (g) fluorescent images of the particle mixture in the hemocytometer before and after separation.

In order to compare and validate the viscoelastic focusing, we suspended both the 5-µm and 13-µm particles mixture in the conventional aqueous ferrofluid which has the same volume ratio of magnetite nanoparticles (0.11%). We tested their separation performance in the same microfluidic device at the flow rate of 15 µl/min. Particles randomly enter the inlet (Figure 6a), and still occupy a wide region of channel width even after 20 mm in the square channel because there is not any intrinsic or external force to focus particles (Figure 6b). When the wide particle streaks enter the magnetic region, 13-µm particles (red fluorescence) are repelled from the magnet due to the intense negative magnetophoretic force. However, the magnetophoretic force is too weak on 5-µm particles (green fluorescence) to deflect them, and they retain as a wide stripe (Figures 6c and 7a). At the outlet region, we can see that 5-µm particles exit from all the three outlets, hindering the efficient separation of particles (Figure 6d).

In contrast, for microparticles in the PEO9-based ferrofluid, microparticles are confined to a tight lateral position before the magnetic region, and then the distance of binary particle streaks is big enough in the downstream for effective separation, Figure 7b. This further demonstrates the superior advantages of PEO-based ferrofluid in the manipulation of microparticles, and we believe that the proposed PEO-based ferrofluid will bring more flexibility and versatility in the manipulation of microparticles. For example, in particle viscoelastic focusing, there are a few particles located at the four corners at relatively low flow rates. By patterning magnets symmetrically along two sides of the microchannel, negative magnetophoretic forces will not only speed up the viscoelastic centreline focusing, but also may eliminate the particle elastic focusing positions at four corners of the cross section. In addition, negative magnetophoresis can be used to confine particles along one sidewall of channels, and in the downstream, the elastic force with its magnitude proportional to the cubic of particle diameter \(a^3\) can enrich a size-dependent separation of microparticles. Furthermore, it would be very intriguing to couple magnetophoresis and elastic forces together to explore their complex interaction by delicately designing a microfluidic device to
regulate their magnitude in the same order. Since there is no explicit expression to calculate the magnitude of elastic force, it brings the opportunity to utilise the known magnetophoretic force to probe the elastic force. The proposed PEO-based ferrofluid has a wide range of potential applications on manipulation and separation of blood cells, cancer cells and bacteria. For example, separation of circulating tumour cells (CTCs) from peripheral blood of cancer patients is one of promising future directions using the PEO-based ferrofluid. CTCs are the rare cancer cells that escape from solid tumours and enter the bloodstream, and they can be used as a real-time “liquid biopsy” for prognosis monitoring and personalised therapy. CTCs cells usually have a much larger size (≈20 µm in diameter) than most of the normal blood cells (erythrocytes ≈6-8µm in diameter and leukocytes ≈12µm in diameter), therefore separation and enrichment of CTCs using PEO-based ferrofluid is attractive due to the advantages of label-free, sheathless and simplicity. Because CTCs are extremely rare in blood circulation at a concentration of 1-100 CTCs per ml of blood, the throughput of the device needs to be scaled up to satisfy the volume scale (~ml) of blood sample. Meanwhile, biocompatibility of the PEO-based ferrofluid is another concern when adopting it on cellular manipulation. Although PEO solution and ferrofluid were both claimed biocompatible, it is still a lack of systematic study on their influence on cellular viability and functionality.

Figure 6 Particle magnetophoretic deflection in the microfluidic channel using the conventional aqueous ferrofluid. Particle trajectories at (a) inlet; (b) expand region; (c) magnet region; and (d) outlet region. Within these images, (i) bright field images; (ii) and (iii)
fluorescent trajectories of 5-µm and 13-µm particles respectively; and (iv) merged fluorescent images of trajectories of 5-µm and 13-µm particles.

Figure 7 Normalized particles fluorescence profiles at the channel outlet for particles mixture in the conventional aqueous ferrofluid and the PEO-based ferrofluid. The fluorescence intensity profiles are normalized by $S = \int_0^1 y \, dx = 1$.

Conclusions
In this work, we proposed and developed a novel viscoelastic ferrofluid by replacing the Newtonian base medium of the conventional ferrofluid with non-Newtonian viscoelastic PEO solution. And the properties of both viscoelastic centreline focusing and negative magnetophoresis were demonstrated and investigated. By employing both viscoelastic and magnetic properties of the novel ferrofluid in a serial fashion, continuous and sheathless separation of non-magnetic microparticles based on particle size was achieved. In order to
validate and compare the differences, we also tested the separation of non-magnetic particles using the conventional Newtonian ferrofluid. Experimental results demonstrated and ascertained the advantages of the viscoelastic ferrofluid. We envisaged that this novel viscoelastic ferrofluid is expected to bring more flexibility and versatility into the design and functionality in microfluidics.

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