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Ferro-microfluidic device for pathogen detection

Birgit Fischer[1*], Leidong Mao[1#], Mustafa Gungormus[2**], Candan Tamerler[2, 3], Mehmet Sarikaya[2], Hur Koser[1]

[1] *Electrical Engineering, Yale University, USA*
[2] *Material Science and Engineering, University of Washington, USA*
[3] *Melecular Biology and Genetics, Istanbul Technical University, Turkey*

***Abstract* — In a previous work, we demonstrated that traveling wave excitations from integrated electrodes can continuously pump magnetic liquids within a microfluidic channel [1]. The optimum excitation frequency of this pumping is strongly dependent on the hydrodynamic size of the magnetic nanoparticles, and the effect can be used to detect whenever a molecule or pathogen binds to the magnetic nanoparticles within a ferrofluid. Here, we demonstrate the bio-functionality and pathogen detection capability of a ferrofluid comprised of cobalt-ferrite-silica nanoparticles through the use of biotinylated genetically engineered peptides for inorganics (GEPI's) [2]. These biotinylated GEPI's are specifically engineered to attach to the silica surface of the magnetic nanoparticles. Binding of streptavidin to the biotinylated GEPI's on the surface of the magnetic nanoparticles shifts the optimum pumping frequency by an amount that corresponds to the increase in the hydrodynamic size of the nanoparticle. The combination of GEPI-enhanced ferrofluids with integrated microfluidic devices finally enables the development of highly sensitive, portable and cheap pathogen sensor chips.**

Keywords — Colloidal stability, genetically engineered peptides, magnetic liquid, microfluidics, pathogen detection

I. Introduction

Ferrofluids are colloidal suspensions comprised of nanosized magnetic particles that are stabilized with a surfactant in a carrier liquid [3]. Their unique characteristic of combining liquid and magnetic material properties has led to a variety of industrial and biomedical applications [4]. The applications in the field of biomedicine are particularly broad in scope, and include drug delivery [5], hyperthermia therapy [6], magnetic resonance imaging (MRI) contrast agents [7], pathogen sensors [1, 8], as well as cellular sorting and manipulation [9,10]. For biomedical applications, the colloidal stability of the ferrofluid and its capability to be rendered biofunctional are critical.

Magnetic particles within a ferrofluid will respond to externally applied DC magnetic fields by moving to high field gradient locations, but the ferrofluid quickly reaches a static equilibrium. Continuous actuation of a ferrofluid requires AC fields. Recently, we have demonstrated that traveling wave excitations can be used to continuously pump ferrofluids in closed-loop channels, both at the macro and micro-scales [1, 11]. The pumping phenomenon displays a frequency-dependent spectrum that is determined by the overall magnetic relaxation time constant of the particular ferrofluid [1]. The size distribution of the magnetic nanoparticles, in turn, governs the magnetic relaxation dynamics [4].

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Birgit Fischer: for ferrofluid synthesis of this project please contact **birgit.fischer@yale.edu. #Leidong Mao: for microdevice fabrication aspects please contact **leidong.mao@yale.edu.** ** Mustafa Gungormus: for GEPI's selection and fabrication please contact **musgun@u.washington.edu.**

When an external field is applied, the magnetic moment within a small particle aligns with the field by rotating inside the nanoparticle (Néel relaxation). Above a critical size, the magnetic moment stays fixed within the nanoparticle, and the entire particle rotates to align its magnetization to the applied field (Brownian relaxation) [12]. For cobalt-ferrite, this critical particle diameter is about 5 nm; a ferrofluid comprised of cobalt-ferrite nanoparticles larger than this limit relaxes primarily via particle rotation. The characteristic time constant for Brownian relaxation depends on the overall hydrodynamic size of the nanoparticles, including the surfactant layer and any other biological species that may be on their surface. This observation implies that changes in the Brownian time constant may be used to directly monitor binding events on the surface of the magnetic nanoparticles.

In this work, we used both ferrofluid pumping and its AC susceptibility spectrum to monitor changes to the average effective hydrodynamic diameter of the magnetic nanoparticles. We utilized genetically engineered peptides for inorganics (GEPI's) both as an additional surfactant species and to render the magnetic particles biofunctional. These GEPI's (short polypeptides with 7 to 15 amino acids) can be engineered for specific and strong attachment to the surface of various nanomaterials [2], and they can be synthesized as part of various receptor proteins for biomolecular recognition and detection applications [13]. The use of GEPI's in this manner renders ferrofluid functionalization and stabilization simple and flexible. Here, we present the detection of avidin-coated microbeads (used as pathogen stimulants) through ferrofluid pumping and AC-susceptibility measurements on ferrofluids functionalized with biotinylated GEPI's.

II. Materials and Methods

A. Ferrofluid Synthesis

Cobalt-ferrite particles were prepared via a co-precipitation method as described in [14]. Afterwards, the magnetic nanoparticles were stabilized in a colloidal suspension with nitric acid as an intermediary surfactant [15]. To achieve a stable dispersion at neutral pH, the magnetic particles were dialyzed against a 5 mM sodium citrate solution. The particles were then covered with a thin silica shell through a modified Stöber synthesis approach [16]. Finally, the suspension of cobalt-ferrite/silica nanoparticles was dialyzed against deionized (DI) water for one week to yield the final ferrofluid.

B. Chemicals

Avidin-coated microbeads in a borate buffer (pH 8; 1%

Bovine Serum Albumin (BSA) and sodium azide; diameter 3.6 μm) were purchased from Duke Scientific Corporation. The microbeads consist of polystyrene with a copolymer grafted surface and have more than $1x10^6$ biotin binding sites per molecule.

Streptavidin from *Streptomyces avidinii* was purchased from Sigma-Aldrich as an essentially salt-free, lyophilized powder and had ~14 units/mg protein. For the experiment streptavidin was dissolved in deionized water.

C. GEPI's Selection and Sample Preparation

Various approaches are currently used in the literature to obtain polypeptide sequences with specific affinity to inorganic surfaces [2]. Here, we used phage/flagellar display techniques to select polypeptides which strongly bind to the surfaces of silica (determined by surface plasmon resonance spectroscopy [13]). The selection process starts with a large random library of peptides that consist of different amino acid sequences of the same length. These sequences are generated on the surface of phage viruses or within a flagellar protein of bacteria. A heterogeneous mixture of recombinant phages or bacteria is then contacted with the inorganic substrate. The unbound organisms are eventually washed away and individual clones are selected. Finally, the eluted organisms are amplified and the strong-binding polypeptides are sequenced, synthetically prepared and functionalized.

For both AC susceptibility and pumping experiments, cobalt-ferrite/silica ferrofluid with no additional GEPI's (Sample A) was used as control. For the experiments, GEPI's – either biotinylated (Sample B) or bare (Sample C) – were added to the cobalt-ferrite/silica ferrofluid in a PBS solution and incubated for at least 15 minutes, before adding the avidin-coated microbeads dispersion or streptavidin solution.

D. Microfluidic Device Fabrication

Microfluidic devices were prepared by conventional micro-fabrication methods and soft-lithography, as described in [1]. Briefly, a set of parallel electrodes were obtained by wet ething of metal-clad printed circuit boards (PCB's). Once patterned, the electrodes were wire-bonded in quadrature to create a traveling wave excitation within the channel [11]. Pressure sensors connected to both ends of the microfluidic channel monitored the pumping pressure. The pumping spectrum was automatically recovered and plotted via a Labview program. A schematic overview of the experimental setup is given in Fig. 1.

E. AC-Susceptibility Setup

Susceptibility measurements were performed using a LCR meter from Agilent (model E4980A). A pick-up coil was inserted into a field coil. From the difference of the mutual inductivity of coils with and without a ferrofluid inserted, the apparent susceptibility at different frequencies could be directly extracted.

III. Experiments and Results

A. Characterization of the Cobalt-Ferrite/Silica Ferrofluid

A cobalt-ferrite/silica ferrofluid was prepared as described above. In transmission electron microscopy (TEM) images, the cobalt-ferrite cores of the nanoparticles were found to range in size from 5 nm to 20 nm (Fig. 2). The silica shell thickness was around 4 nm. Within this size range, virtually all the nanoparticles in the ferrofluid were expected to relax through the Brownian mechanism under applied magnetic fields.

B. Ferrohydrodynamic Pumping

Fig. 3 depicts the ferrohydrodynamic pumping pressure signal from the microfluidic device as a function of frequency. The frequency at which the pumping signal is maximum is determined by the overall magnetic relaxation time constant of the ferrofluid. As such, the location of the ferrohydrodynamic pumping peak in frequency is a measure of the average hydrodynamic diameter of the magnetic particles. The polydispersity of the magnetic nanoparticles results in a broadening of the pumping spectrum. Anything that binds to the surface of the magnetic particles increases their hydrodynamic diameter and shifts the peak location to lower frequencies. In this fashion, the microfluidic device may be used as a cost-effective and portable pathogen sensor [1, 8].

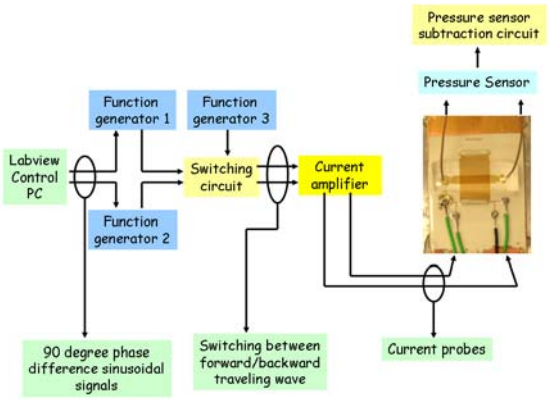

Figure 1. Setup for the ferrohydrodynamic pumping experiments.

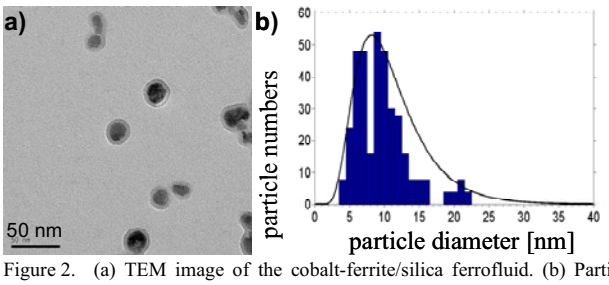

Figure 2. (a) TEM image of the cobalt-ferrite/silica ferrofluid. (b) Particle size distribution of the cobalt-ferrite core determined from TEM images.

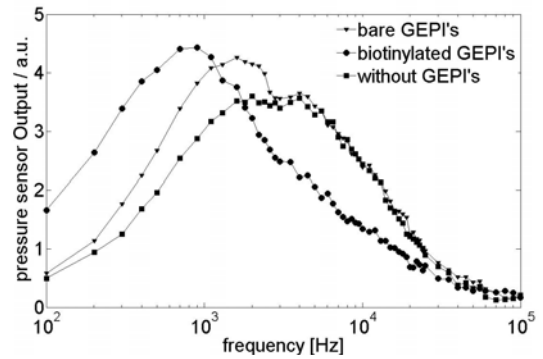

Figure 3. By adding avidin-coated microbeads, the pumping spectrum of the ferrofluid with biotinylated GEPI's shifts to lower frequencies. With bare GEPI's, an increase of the pumping amplitude for low frequencies only. The microbead concentration in all samples is about 66400/cc.

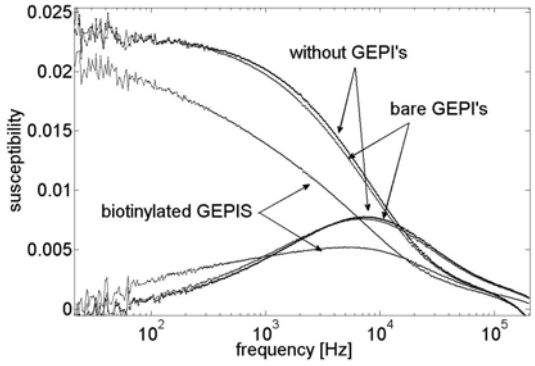

Figure 4. Frequency dependent susceptibility spectra of cobalt-ferrite/silica ferrofluid samples; the lower curves depict the imaginary component of the AC-susceptibility, while the higher curves correspond to the real component. The presence of avidin-coated microbeads within the ferrofluid shifts the imaginary part of the AC-susceptibility for the ferrofluid with biotinylated GEPI's, whereas the two controls (ferrofluid samples with bare GEPI's or no GEPI's at all) lie on top of each other. The microbead concentration in all samples is about 66400/cc.

In actual experiments, avidin-coated microbeads were added to all three ferrofluid samples. While the sample with no GEPI's (Sample A) showed no response to the microbeads, the sample with bare GEPI's (Sample C) displayed an increase in pumping signal at low frequencies (Fig. 3). This phenomenon could be attributed to some degree of non-specific binding between bare GEPI's and the microbeads; near the optimal pumping peak frequency, shear forces disrupt this interaction, so that the particles rotate again without the beads, and the pumping signal continues to trace that of the control ferrofluid. On the other hand, the sample with biotinylated GEPI's (Sample B) displayed a clear shift in the pumping peak location towards lower frequencies.

C. AC-Susceptibility Measurements

AC-susceptibility setup measures the overall relaxation of the magnetic moment within the cobalt-ferrite particles. As in the ferro-microfluidic pumping device, only Sample B is expected to show a signal shift that corresponds to the presence of the avidin-coated microbeads. In the case of AC-susceptibility, the corresponding signal is the imaginary part of the susceptibility spectrum. Fig. 4 depicts the real and imaginary parts of this spectrum for the cobalt-ferrite/silica ferrofluid for the three samples described above. As expected, only the sample with biotinylated GEPI's (Sample B) responds to the microbeads. For that sample, a shift to lower frequencies (indicating larger average nanoparticle diameters) is clearly visible. A systematic study of the susceptibility spectra as a function of bead concentration reveals that as few as 8300 microbeads/cc could clearly be observed (Fig. 5). The absolute minimum concentration that is detectable is probably much smaller, and will be characterized in detail as part of our future work.

It is interesting to note here that, compared to the peak frequency locations in the susceptibility data the optimal pumping peaks from the microfluidic device are lower in frequency (Fig. 6). This observation implies that the ferro-microfluidic device may be causing structure formation within the ferrofluid, due to the relatively high magnetic field gradients generated over the electrodes. Pressure sensors with higher sensitivity should enable the reduction in the currents applied for ferrohydrodynamic actuation, and help to reduce structure formation within the ferrofluid.

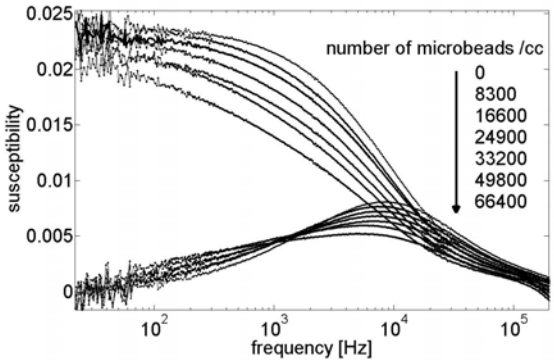

Figure 5. Increasing the concentration of avidin-coated microbeads within the ferrofluid systematically shifts the imaginary part of the AC-susceptibility spectrum to lower frequencies.

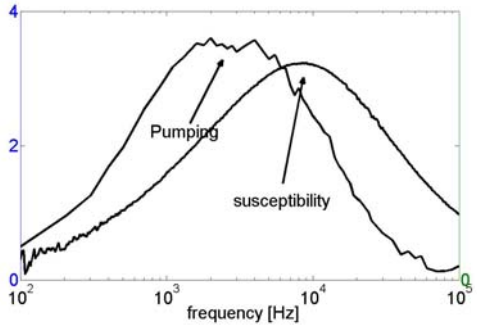

Figure 6. Comparison between the signals from ferrohydrodynamic pumping and AC-susceptibility. The optimal pumping frequency is lower than the maximum in the imaginary susceptibility, indicating structure formation within the ferrofluid under high magnetic field gradients from the electrodes of the microfluidic device.

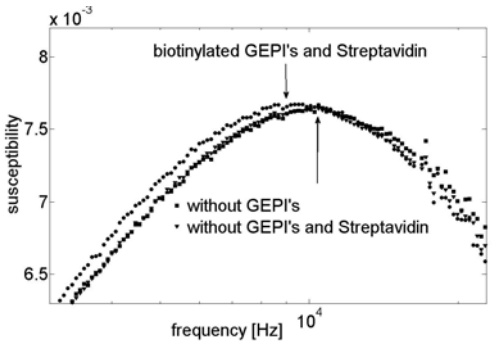

Figure 7. Imaginary part of the susceptibility of cobalt ferrite/silica ferrofluid with biotinylated GEPI's after adding streptavidin (20 μg/cc). As a control, streptavidin is added by the same amount to the sample with no GEPI's. The spectra are compared to that from a ferrofluid sample with no streptavidin.

D. Detection of Small Molecules

The enabling mechanism for ferrofluid functionalization involves biotinylated GEPI's. In order to test their efficacy and the performance limits of the overall ferrofluid system, we have added individual streptavidin molecules into the ferrofluid. Streptavidin has a hydrodynamic size of just less than 5 nm [17]. Binding of streptavidin to the surface of cobalt-ferrite/silica nanoparticles through biotinylated GEPI's has indeed been observed through initial AC-susceptibility measurements (Fig. 7).

IV. CONCLUSION

In this study, we introduced genetically engineered peptides for inorganics (GEPI's) as a new type of surfactant for cobalt-ferrite/silica nanoparticles to create a stable and bio-compatible ferrofluid. These GEPI's allow the specific functionalization of our ferrofluids without any complicated post-synthesis steps. Once the ferrofluid is synthesized, functionalization is as simple as mixing two solutions. We show that such a functionalization is indeed feasible and implementable by demonstrating, both through micro-pumping and AC-susceptibility experiments, that such functionalized ferrofluids can be used to detect simulated pathogens.

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