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Formation and properties of magnetic chains for 100 nm nanoparticles used in separations of molecules and cells

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ABSTRACT

Optical observations of 100 nm metallic magnetic nanoparticles are used to study their magnetic field induced self assembly. Chains with lengths of tens of microns are observed to form within minutes at nanoparticle concentrations 10^{10}/mL. Chain rotation and magnetophoresis are readily observed, and SEM reveals that long chains are not simple single particle filaments. Similar chains are detected for several 100 nm commercial bio-separation nanoparticles. We demonstrate the staged magnetic condensation of different types of nanoparticles into composite structures and show that magnetic chains bind to immuno-magnetically labeled cells, serving as temporary handles which allow novel magnetic cell manipulations.

Magnetic nanoparticles are widely used in biology and medicine for applications which include biomolecule purification and cell separation [1], magnetic resonance imaging (MRI) contrast agents [2], and bio-magnetic sensors [3]. There is also much recent interest in magnetic hyperthermia [4] and magnetically targeted delivery [5] and transfection [6]. In all of these applications the magnetic field induced aggregation of particles can have strong effects by altering interparticle distances and creating clusters which are subject to size- and shape-dependent forces. Understanding the basis of cluster formation and the resulting modifications to composite particle behavior is therefore important to a broad range of phenomena. Although the formation and properties of magnetic chains are well documented for micron-sized particles [7], direct real space observation of particle assembly becomes difficult when particle diameters are reduced to the nanometer scale. As a result, even the existence of magnetic chains can become uncertain.

We, therefore, initiated studies to observe the formation and properties of magnetic chains composed of 100 nm metallic magnetic nanoparticles. Our initial interests included the basic formation of chains and their magnetophoretic velocities and viscous drag forces, which are helpful for understanding the effects of chaining on magnetic separation and retention. In the course of these studies, we found that we could observe chains in many commercial 100 nm magnetic reagents, including MagCellect (MC) [8] (Immunicon, R&D systems), 130 nm diameter Nanomag D [9] (microMod) and Feridex [10] (Berlex). Each of these types of nanoparticles has high iron oxide content and diameters near 100 nm. We examined mixtures of MagCellect and synthetic antiferromagnetic (SAF) nanoparticles [11], which exhibit chain formation at different magnetic fields, to show that composite structures, which involve magnetically staged condensation of both types of nanoparticles, can be obtained. Finally, we observed that chains magnetically reversibly bind to immuno-magnetically labeled cells, and can enhance the cell's magnetic responsiveness and allow novel magnetic manipulations.

The optical observations of individual magnetic nanoparticles that are reported here were enabled by our development of a novel type of highly magnetic nanoparticle [11], which is fabricated from pure metals and strongly scatters light. These monodisperse synthetic antiferromagnetic nanoparticles were made using layered metal films deposited on substrates which are pre-patterned using nanoimprint lithography. Details of fabrication, magnetic characteristics, and electron microscopy observations of dimensions and layer structure are available elsewhere [11–14]. The layer structure of the 100 nm diameter metal nanoparticles used in this work is 5 Ta/2 Ru/10 $Co_{90}Fe_{10}$/2.5 Ru/10 $Co_{90}Fe_{10}$/2 Ru/5 Ta, where the notation gives the thickness, in nm, and elemental composition of the layers. Tantalum is used as a protective layer, ruthenium is used to control intra-layer magnetic interactions, and a cobalt iron alloy provides high magnetic moment (1500 emu/cc). These SAF exhibit zero remanence, a linear low-field response, and adjustable magnetic saturation fields. The magnetic moment of the particles is

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deliberately made comparable to those of 100 nm spherical iron oxide particles by using thin $Co_{90}Fe_{10}$ layers.

These nanoparticles can be directly viewed using reflected light optical microscopy with a high numerical aperture (NA) immersion objective lens, even though the nanoparticle size is well below the diffraction limit. The high NA requirement results in a focal depth of field of only a few microns, and optical working distances near 100 μm require thin sample cells. Thus, these measurements are performed with immersion or on liquid samples between a cover slip and, typically, a 20 μm deep chamber slide (Hamilton–Thorne). Beneath the slide is a 1 in diameter, 1 in long cylindrical Alnico permanent magnet, magnetized along the cylinder axis, which can be rotated and translated relative to the slide. When the magnet is horizontal and centered under the objective, the magnetic field is also horizontal and has no horizontal gradient. Horizontal gradients are obtained by translating the magnet off center. In this geometry [15], there is always a vertical field gradient, and particles always seek the high fields produced near the magnet. All images were extracted from videos which may be viewed [15].

For studies of cell–chain interactions, human umbilical vein endothelial cells (HUVEC) [16] were grown from frozen samples through several generations using EDTA-mediated passages on 4 in culture plates. Harvested cells were washed repeatedly using 300 g centrifugation for 5 min to form pellets, followed by aspiration and resuspension in PBS buffer. The washed HUVEC were then labeled with biotinylated antibody, as per the supplier's protocol, using a PlusCellect kit (R&D Systems) targeting platelet endothelial cellular adhesion molecule (PECAM1, CD31) surface markers. After this incubation, streptavidin-coated MagCellect nanoparticles were added for a second incubation, after which the labeled cells were washed repeatedly to remove excess antibody and nanoparticles. To study chain–cell interactions, magnetic nanoparticles were added back to the washed labeled cell sample, or washing steps were omitted.

Single 100 nm metal particles can be easily tracked within an optical cell provided their vertical range is small enough that diffusing particles cannot leave the few micron focal depth. In deeper cells these nanoparticles, with diffusion constants 2 $\mu m^2/s$, move through the focal depth in about a second and appear as flickering dots in image streams [15] captured with 40 ms exposures and 2 Hz frame rates. SEM and optical counting of particles confirm that most SAF are present as individual particles when no magnetic field is applied. Fig. 1a shows an image of these diffusing nanoparticles in zero field at a concentration 10^{10}/mL. When the magnetic field is increased to 1 kOe, the nanoparticles magnetize and initially coalesce into short linear chains due to attractive interparticle magnetic dipole interactions. Diffusion slows as the chains extend and continue to coalesce, primarily at chain ends. Fig. 1b shows chains formed after 50 s of field exposure at concentrations 10^{10}/mL. The chains are all located on the slide surface, and no free particles remain in solution. The vertical confinement arises from the vertical gradient of the magnetic field, which results in an equilibrium concentration [16,17] of the form $C(z)\sim\exp(-z/z_o)$ where $z_o = k_BT/(N\ m\nabla H)$. Here, the energy has been expanded to first-order in the z coordinate, k_BT ($\sim 4\times10^{-14}$ ergs) is the Boltzmann energy, m is the nanoparticle moment ($\sim10^{-13}$ emu), ∇H ($\sim$1 kOe/cm), and N the

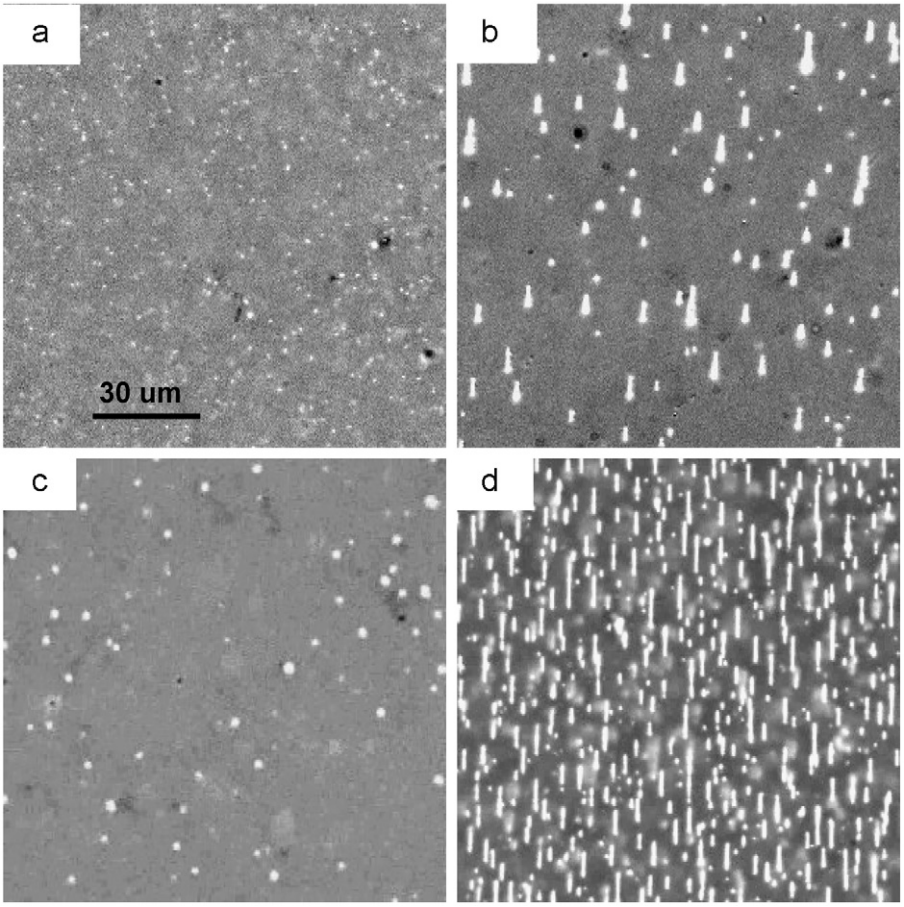

Fig. 1. (a) Images of SAF nanoparticles in a 20 μm deep chamber slide in zero field and at 10^{10}/mL initial concentration. (b) Chain structures formed 50 s after H was increased to 1 kOe, 10^{10}/mL. (c) Small chains which form under the same conditions, except the concentration is 10^9/mL. (d) Numerous chains formed in 15 s when the concentration is 10^{11}/mL.