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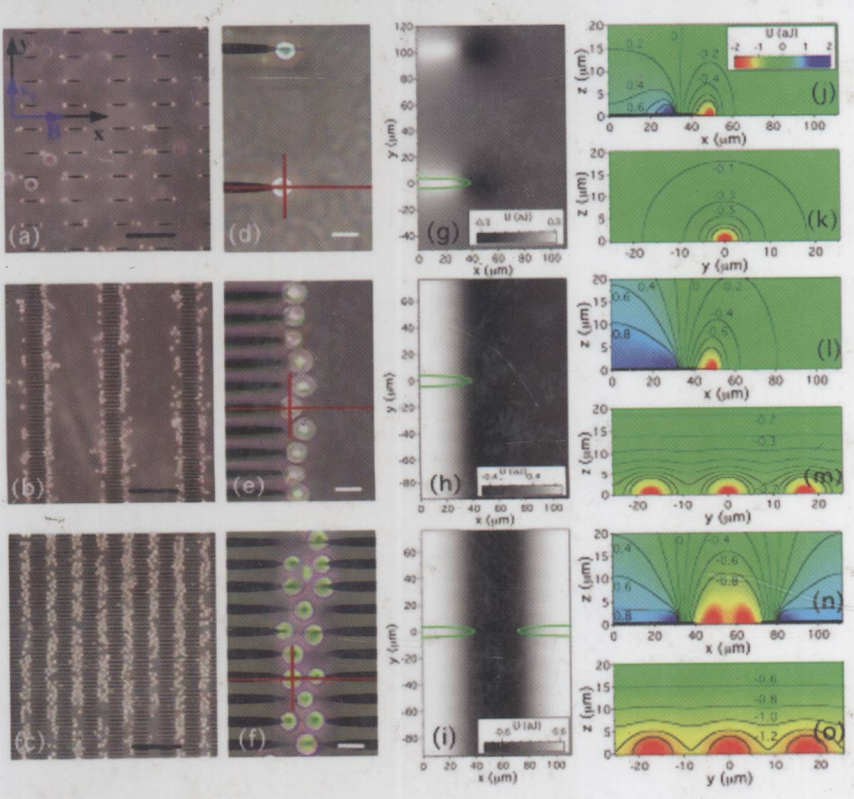
Biomedical Applications of Nanotechnology

Edited by
Vinod Labhasetwar and Diandra L. Leslie-Pelecky



BIOMEDICAL APPLICATIONS OF NANOTECHNOLOGY

EDITED BY

Vinod Labhasetwar
Department of Biomedical Engineering
Lerner Research Institute
Cleveland Clinic, Cleveland, Ohio

Diandra L. Leslie-Pelecky
Department of Physics and Astronomy
University of Nebraska—Lincoln

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BIOMEDICAL APPLICATIONS OF NANOTECHNOLOGY

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PREFACE

Nanotechnology is poised to make potentially revolutionary innovations in areas of biomedical science such as diagnostics, drug therapy, and imaging. In the future, nanotechnology using different biomarkers will be able to diagnose patients in much earlier stages of disease. Microchip-based diagnostic tests using biomarkers conjugated to nanoparticles or quantum dots can detect abnormalities at molecular levels that potentially can lead to disease progression. Nanotechnology can overcome anatomical and physiological barriers to deliver drugs more effectively to the target sites to reduce nonspecific effects. Many drugs, especially modern therapeutics, cannot be successful unless mechanisms for their effective delivery are developed. Nanotechnology can be a powerful tool to address delivery-related issues such as poor solubility or stability in biological environments. Imaging plays an important role in detection of pathologies such as tumors or vascular pathologies. Magnetic nanoparticles are under extensive investigation to enhance and improve the magnetic resonance imaging (MRI) capability for early detection of diseases.

Researchers in this area realize that the field of nanotechnology has matured over the last two decades of extensive research. We have developed the ability to design new systems, smart bioresponsive polymers that respond to changes in the bioenvironment stimulated by disease conditions, and we have a better understanding of their action mechanisms, interactions with cells and tissue, body distribution, and clearance. Also, we know how to assemble biomolecules into different nanostructures. We appreciate the pros and cons of each system and are making every effort to refine them to further enhance their therapeutic potential. The progress in the field of nanotechnology is evident from the range of nanotechnologies under various stages of clinical development from diagnostic to drug delivery applications. The field has certainly galvanized interdisciplinary research by bringing together polymer science, biology, pharmaceutical sciences, medicine, and physical science. Collaborative efforts address issues from various angles, and they may develop more effective solutions.

As we continue exploring nanotechnology for biomedical applications, it is essential for us to ensure that the nanotechnologies developed are safe. Nanotoxicity is an emerging field of research that will become an integral part of nanotechnology research; however, the burden for ensuring the safety of these technologies resides with all of us. We are pleased to cover some of the above important aspects of nanotechnology in this book.

Cleveland, Ohio
Lincoln, Nebraska
March 2007

VINOD LABHASETWAR
DIANDRA L. LESLIE-PELECKY

CONTRIBUTORS

Samuel D. Bader, Materials Science Division and Center for Nanoscale Materials, Argonne National Laboratory, Argonne, Illinois

Liaohai Chen, Biosciences Division, Argonne National Laboratory, Argonne, Illinois

Seok-Hwan Chung, Materials Science Division and Center for Nanoscale Materials, Argonne National Laboratory, Argonne, Illinois

Edward J. Felton, Department of Physics and Astronomy, Johns Hopkins University, Baltimore, Maryland

Axel Hoffmann, Materials Science Division and Center for Nanoscale Materials, Argonne National Laboratory, Argonne, Illinois

Sarah E. Johnson, Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska

Raghuraman Kannan, Director, Nanoparticle Product Core Facility, Department of Radiology, University of Missouri, Columbia, Missouri

Rangaramanujam M. Kannan, Department of Chemical Engineering and Material Science and Biomedical Engineering, Wayne State University, Detroit, Michigan

Sujatha Kannan, Critical Care Medicine, Department of Pediatrics, Children's Hospital of Michigan, Wayne State University, Detroit, Michigan

Kattesh V. Katti, Nanoparticle Product Core Facility, Department of Radiology, University of Missouri, Columbia, Missouri

Mahima Kaushik, Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska

Irine Khutsishvili, Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska

Robert H. Kraus, Jr., Biophysics and Quantum Physics Group, Los Alamos National Laboratory, Los Alamos, New Mexico

Vinod Labhasetwar, Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio

Diandra L. Leslie-Pelecky, Department of Physics and Astronomy, Nebraska Center for Materials Nanoscience and Nanoscience, University of Nebraska—Lincoln, Lincoln, Nebraska

Souvik Maiti, Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska

Lee Makowski, Biosciences Division, Argonne National Laboratory, Argonne, Illinois

Luis A. Marky, Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska

Olga Mykhaylyl, Institute of Experimental Oncology, Technische Universität München

Chris Olsen, Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska

Omathanu P. Perumal, Omathanu P. Perumal College of Pharmacy, South Dakota State University, Brookings, South Dakota

Christian Plank, Institute of Experimental Oncology, Technische Universität München, Munich, Germany

Maram K. Reddy, Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio

Daniel Reich, Department of Physics and Astronomy, Johns Hopkins University Baltimore, Maryland

Ulrike Schillinger, Institute of Experimental Oncology, Technische Universität München, Munich, Germany

Ronald Shikiya, Department of Pharmaceutical Science, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska

Dialekti Vlaskou, Institute of Experimental Oncology, Technische Universität München, Munich, Germany

Bradford Wright, Biophysics and Quantum Physics Group, Los Alamos National Laboratory, Los Alamos, New Mexico

Murali Mohan Yallapu, Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio

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1

BIOLOGICAL APPLICATIONS OF MULTIFUNCTIONAL MAGNETIC NANOWIRES

Edward J. Felton and Daniel H. Reich

1.1 INTRODUCTION

Nanoscale magnetic particles are playing an increasingly important role as tools in biotechnology and medicine, as well as for studying biological systems. With appropriate surface functionalization, they enable the selective application of magnetic forces to a wide range of cells, subcellular structures, and biomolecules, and have been applied to or are being developed for areas including magnetic separation, magnetic biosensing and bioassays, drug delivery and therapeutics, and probes of the mechanical and rheological properties of cells [1–10]. Despite these successes, however, the structure of the magnetic particles in common use limits the range of potential applications. Most biomagnetic particles available today are spherical, with either (a) a "core-shell" structure of concentric magnetic and nonmagnetic layers or (b) magnetic nanoparticles randomly embedded in a nonmagnetic matrix [2, 11]. These geometries constrain the range of magnetic properties that can be engineered into these particles, as well as their chemical interactions with their surroundings, because such particles typically carry only a single surface functionality. A new and more versatile approach is to use asymmetric, multisegment magnetic nanoparticles, such as the metal nanowires shown in Figure 1.1.

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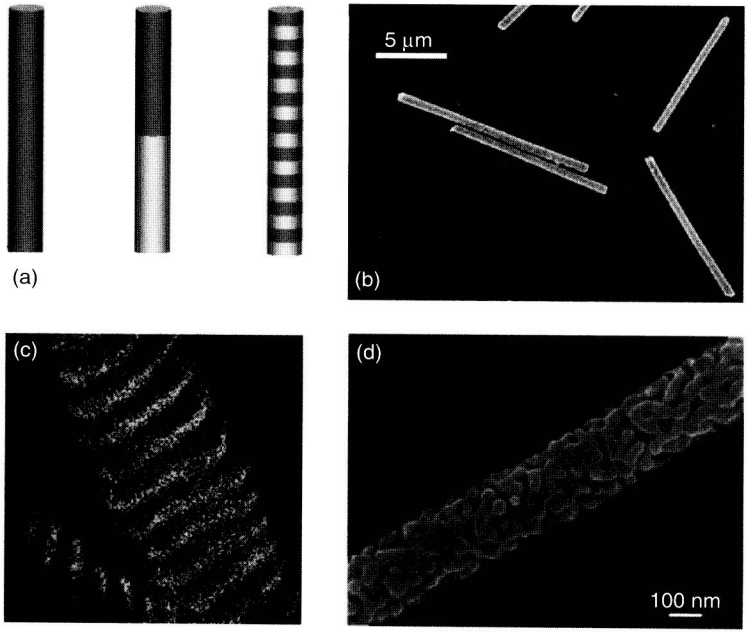

Figure 1.1. (a) Schematic illustration of magnetic nanowires, showing single-segment, two-segment, and two-component multisegment nanowires. (b) SEM image of 15 μm Ni nanowires (from Ref. 24, reproduced with permission of The Royal Society of Chemistry). (c) EELS image of Ni–Cu multisegment nanowires (reprinted with permission from Ref. 15, Copyright 2003, American Institute of Physics). (d) Nanoporous Au–Ag nanowire with Ag etched away. (Reprinted with permission from Ref. 16. Copyright 2003 American Chemical Society.)

The multisegment architecture of these particles, along with the ability to vary the aspect ratio and juxtaposition of dissimilar segments, allows the nanowires to be given a wide range of magnetic, optical, and other physical properties. In addition, differences in the surface chemistry between segments can be exploited to selectively bind different ligands to those segments, enabling the development of magnetic nanoparticle carriers with spatially resolved biochemical functionality that can be programmed to carry out multiple tasks in an intracellular environment.

This chapter provides an overview of recent results of a research program, centered at Johns Hopkins University, that is aimed at development of multifunctional magnetic nanowires for biotechnology applications. Section 1.2 provides a brief introduction to the fabrication process, and this is followed in Section 1.3 by an overview of the physical properties of the nanowires that are important in a biotechnological context. Sections 1.4–1.6 describe our development of the needed "tool-kit" for biological applications: manipulation of the nanowires in suspension, chemical functionalization, and self-assembly techniques. Section 1.7 discusses prospects for magnetic biosensing using nanowires, and Sections 1.8 and 1.9 discuss the major biological applications of

the nanowires explored to date: novel approaches to magnetic separations, new tools for cell positioning and patterning, and new carrier particles for drug and gene delivery.

1.2 NANOWIRE FABRICATION

Nanowires are fabricated by electrochemical deposition in nanoporous templates. Originally developed for fundamental studies of the electrical and magnetic properties of modulated nanostructures [12], this method offers control of both nanowire size and composition and thus allows the nanowires' magnetic and chemical properties to be tailored for specific biological applications. To make the nanowires, a copper or gold conductive film is sputtered on one side of the template to create the working electrode of a three-electrode electrodeposition cell. Metal is then deposited from solution into the template's pores to form the wires. The nanowires' diameter is determined by the template pore size and can range from 10 nm to approximately 1 μm. The wires' length is controlled by monitoring the total charge transferred and is only limited by the thickness of the template. After the nanowire growth is complete, the working electrode film is etched away and the template is dissolved, releasing the nanowires into suspension.

Ferromagnetic nickel nanowires were commonly used in the work reported here. Grown in commercially available 50 μm-thick alumina templates, they have a radius of 175 ± 20 nm and lengths ranging from 5 to 35 μm. An SEM image of 15 μm-long nickel nanowires is seen in Figure 1.1b. The high pore density of the alumina templates (3×10^8 cm^{-2} [13]) enables fabrication of large numbers of nanowires. In addition to single-component nanowires such as these, nanowires comprised of multiple segments can be made by changing the deposition solution during growth. This technique has been used with alumina templates to create two-segment Ni–Au nanowires [14]. Alternatively, multisegment nanowires of certain materials can be grown from a single solution by varying the deposition potential. One example is the alternating ferromagnetic and nonmagnetic layers of the Ni–Cu nanowire shown in Figure 1.1c [15]. Nanowires incorporating two metals can also be synthesized as alloys. In one example, this technique has been used to produce high-surface area nanoporous Au wires by selectively etching away the Ag from Au–Ag alloy nanowires, as shown in Figure 1.1d [16].

1.3 PHYSICAL PROPERTIES

The elongated architecture of the nanowires and the flexibility of the fabrication method permit the introduction of various magnetic and other physical properties. The magnetic properties can be tuned and controlled through the size, shape, and composition of magnetic segments within the wires. For example, due to their high magnetic shape anisotropy, single-segment magnetic nanowires form nearly single-domain states with large remanent magnetizations for a wide range of nanowire lengths. This is illustrated in Figure 1.2, which shows magnetic hysteresis curves for 175 nm-radius nickel nanowires of different lengths [17]. The shape of the hysteresis curves is

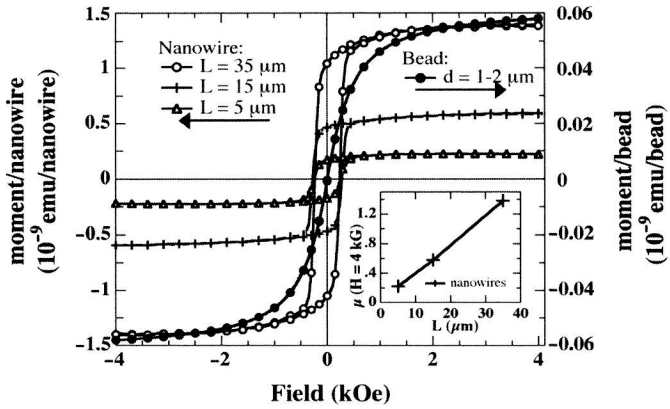

Figure 1.2. Room temperature magnetization versus field curves for 1 to 2 μm beads, 5, 15, and 35 μm nanowires. Inset: Saturation moment versus nanowire size. (Reprinted with permission from Ref. 17. © 2004 IEEE.)

nearly independent of nanowire length, with coercive field $H_C \sim 250$ Oe and remanent magnetization $M_R \sim 0.8M_S$, where M_S is the saturation magnetization. These large, stable, and well-aligned moments make such nanowires useful for low-field manipulations of cells and biomolecules, as discussed in Section 1.8. As seen in the inset, M_S scales linearly with the wire length, and at high fields the nanowires have moment per unit length $\mu/L = 3.9 \times 10^{-11}$ emu/μm. For comparison, Figure 1.2 also shows the magnetic moment of commercially available 1.5 μm-diameter magnetic beads. Note that while the volume of the longest nanowires shown here is only 1.5 times that of the beads, their high-field moment is 20 times that of the beads. Thus the nanowires can provide significantly larger forces per particle in magnetic separations and other high-field applications.

There are, of course, biomagnetic applications in which large magnetic moments in low field are not desirable. These include situations in which it is important to control interactions among particles to reduce agglomeration in suspension. The remanent magnetization of multisegment nanowires such as those shown in Figure 1.1c can be tuned by controlling the shape of the magnetic segments [15, 18, 19]. If the magnetic segments within a multisegment nanowire have an aspect ratio greater than unity, shape anisotropy favors the adoption of a high-remanence state with the segments' moments parallel to the wire axis, even if they are short compared to the length of the nanowire, as shown in Figure 1.3a. In contrast, if the magnetic segments are disk-shaped (aspect ratio < 1), the shape anisotropy of the individual segments favors alignment of their moments perpendicular to the nanowire axis. Dipolar interactions between the segments then favor antiparallel alignment of the moments of neighboring segments, leading to a low-moment state in zero field, as shown in Figure 1.3b.

In addition to defining the magnetic properties, the segment composition can be exploited for other purposes. For example, the high-surface-area nanoporous gold segments previously mentioned (Figure 1.1d) can be used for efficient chemical functionalization, or for biosensing applications. Optical properties of the nanowires can also

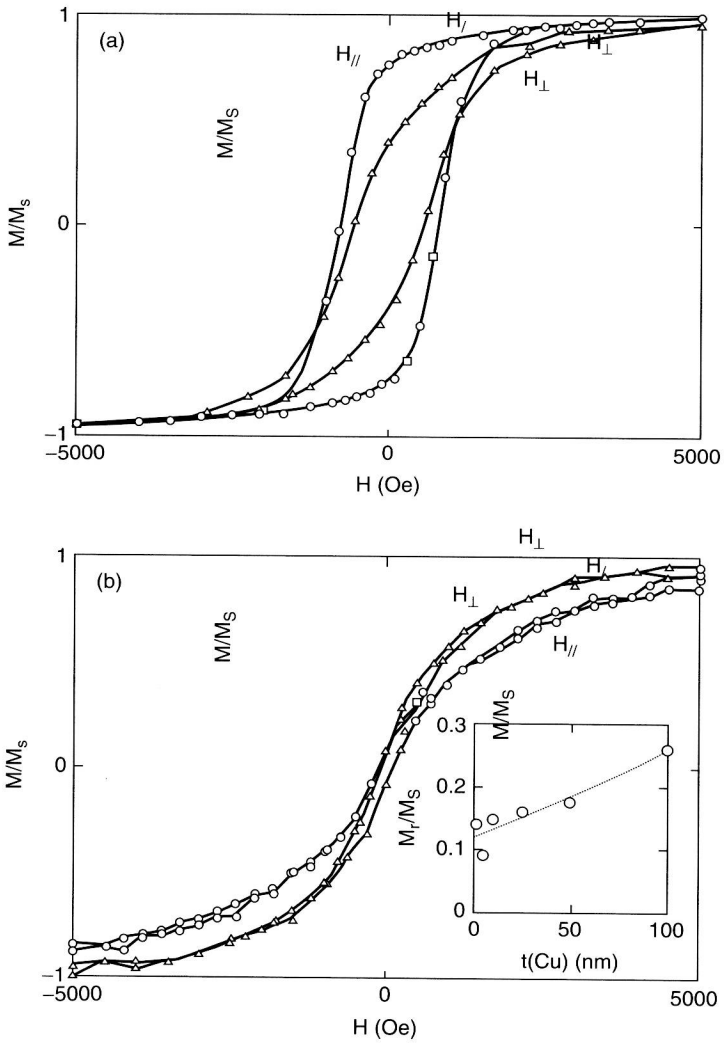

Figure 1.3. Room temperature magnetization versus field curves for arrays of Ni–Cu multilayer nanowires in the template. (a) Ni–Cu nanowires with rod-shaped Ni segments (aspect ratio 2.5) and easy axis parallel to the nanowire axis. (b) Ni–Cu nanowires with disk-shaped Ni segments (aspect ratio 0.1) and easy axis perpendicular to the nanowire axis. The inset shows the remanence for Ni–Cu nanowires with disk-shaped Ni segments as a function of Cu layer thickness. (Reprinted with permission from Ref. 15. Copyright 2003, American Institute of Physics.)

be controlled. Differences in reflectivity in Au–Ag multisegment nanowires are being exploited for "nano-barcoding" of molecules and subcellular structures [20], and oxide segments with intrinsic fluorescence can also be introduced.

1.4 MAGNETIC MANIPULATION OF NANOWIRES

The large and tunable magnetic moments of nanowires allow precise manipulation of molecules and bound cells, with applications ranging from cell separations to two-dimensional cell positioning for diagnostics and biosensing, and to the potential creation of three-dimensional cellular constructs for tissue engineering. The approaches we have developed for these applications take advantage of nanowire–nanowire interactions, as well as their interactions with lithographically patterned micromagnet arrays and external fields. To illustrate these capabilities, we first discuss manipulation of the nanowires themselves.

In liquid suspensions, the nanowires readily orient with their magnetic moments parallel to an applied field. Single-segment and multisegment nanowires with long magnetic segments align with the wire axis parallel to the field, and multisegment wires with disk-shaped segments align perpendicular to the field [15,21]. When magnetized, the nanowires interact through dipole–dipole magnetic forces. Self-assembly of the nanowires can be achieved, either in suspension or by allowing the wires to settle on flat substrates. This process can be controlled by an external field. Without an applied field, the nanowires are randomly oriented in the suspension, and they will assemble into random collections due to the anisotropy of the dipolar interaction. Application of a small field suppresses this random aggregation by prealigning the nanowires parallel to each other. The nanowires then form end-to-end chains as they settle out of solution, as shown in Figure 1.4 [22]. The addition of descending nanowires to chains settled on the substrate can yield chains that extend over hundreds of micrometers.

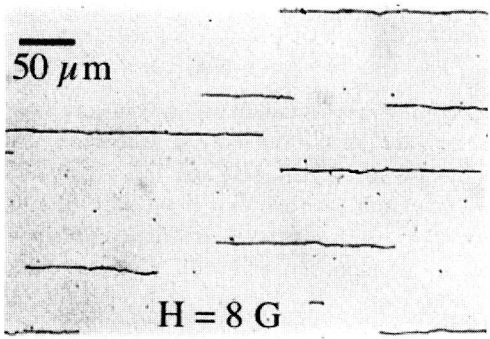

Figure 1.4. Optical micrograph of Ni nanowire chain formation after precipitation from a water suspension in an 8-G external magnetic field. (Reprinted with permission from Ref. 22. Copyright 2002, American Institute of Physics.)

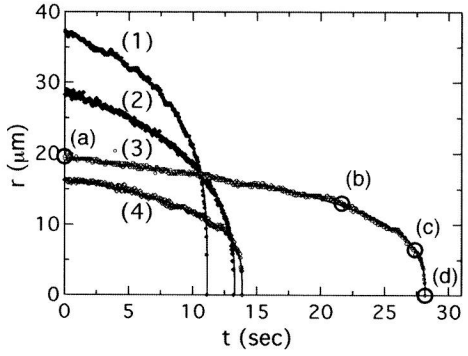

Figure 1.5. Separation versus time for four chain-formation events in a 4-Oe external field. Events (1) and (2) were in water, and events (3) and (4) were in ethylene glycol. (Reprinted from *J Magn Mater*, 249, C. L. Chien et al., Electrodeposited magnetic nanowires: Arrays, field-induced assembly, and surface functionalization, 146–155. Copyright 2002, with permission from Elsevier.)

The motion of both bare nanowires and nanowires bound to cells in suspension is governed by low Reynolds number hydrodynamics, and a nanowire's velocity is given by $\mathbf{v} = \mathbf{F}/D$, where $\mathbf{F}$ is the net force due to external fields, neighboring nanowires, and gravity, and D is the appropriate viscous drag coefficient. Integrating this equation of motion allows precise prediction and modeling of the nanowires' dynamics [21, 23]. For example, Figure 1.5 shows an analysis of a video microscopy study of nanowire chaining dynamics. For all the events shown in Figure 1.5 the wires or chains are nearly collinear. In this case, the force between two wires or chains of lengths L_1 and L_2 is

$$f(r) = -Q_m^2 \left(\frac{1}{r^2} - \frac{1}{(r+L_1)^2} - \frac{1}{(r+L_2)^2} + \frac{1}{(r+L_1+L_2)^2} \right),$$

where r is the end-to-end separation. The nanowires are described very accurately in this and in all subsequent modeling discussed below as extended dipoles with magnetic charges $\pm Q_m = \pm M\pi a^2$ separated by L, where M is the wire's magnetization. The solid curves are fits to $r(t)$ based on the (somewhat involved) analytic form determined from the one-dimensional equation of motion $dr/dt = \tilde{D}f(r)$, where $\tilde{D} = D_1 D_2/(D_1 + D_2)$ is the reduced drag coefficient. Full details are given in Ref. 21. These results demonstrate that quantitative predictions of the nanowire–nanowire interactions and dynamics can be made.

Another important manipulation tool involves using the strong local fields generated by micrometer-size magnetic features patterned by microlithography on substrates to capture and position nanowires and cells [22, 24, 25]. This "magnetic trapping" process works because the nanowires are drawn into regions of strong local field gradients produced by the patterned micromagnets, such as those at the ends of the Ni ellipses shown in Figure 1.6. The snapshots show video frames from a trapping event, and