Electromagnetic needles with submicron pole tip radii for nanomanipulation of biomolecules and living cells

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We describe the design and fabrication of a temperature-controlled electromagnetic microneedle (EMN) to generate custom magnetic field gradients for biomedical and biophysical applications. An electropolishing technique was developed to sharpen the EMN pole tip to any desired radius between 100 nm and 20 μm. The EMN can be used to apply strong static or dynamic forces (>50 nN) to micrometer- or nanometer-sized magnetic beads without producing significant heating or needle movement. Large tip radii (20 μm) allow magnetic force application to multiple magnetic beads over a large area, while small radii (0.1–6 μm) can be used to selectively pull or capture single magnetic beads from within a large population of similar particles. The customizable EMN is thus well suited for micro- and nanomanipulation of magnetic particles linked to biomolecules or living cells. © *2004 American Institute of Physics*. [DOI: 10.1063/1.1802383]

Magnetic micromanipulation of nanometer- and micrometer-sized magnetic particles provides a means to probe molecular binding interactions,[1] separate biological materials,[2] characterize cell mechanical properties,[3–6] and control biochemical activities and gene expression within living cells.[7] Existing magnetic manipulation techniques apply controlled mechanical stress to magnetic particles by generating either large magnetic field gradients to apply directed forces[4–6,8,9] or alternating magnetic field orientations to apply torques.[3] Magnetic methods provide advantages over other techniques, such as optical tweezers, for large-scale separation of materials, manipulation of microparticles, and mechanical analysis of living cells because magnetic gradients can be applied to multiple magnetic particles over much larger distances, and much higher forces can be attained (low nanonewton-level forces with magnetic techniques versus low piconewton forces with optical traps on micrometer-sized beads). Because of their low power requirements, miniaturized electromagnets may also be useful to noninvasively control the position and function of magnetically labeled molecules and cells for applications, such as cell-based biosensors and bioprocessors, as well as directed cell assembly for tissue engineering.

Application of miniaturized electromagnets for molecular and cellular manipulation has been limited by the relatively weak magnetic field gradients, and hence magnetic forces, generated by these devices.[9] An additional problem is the resistive heating of the electromagnet, which can locally denature biomolecules and injure living cells, while also causing thermal expansion of the material used for the electromagnet core. This expansion eliminates precise control over the distance between the magnetic particle and the tip of the electromagnet, thereby hindering control over the precise level of force used to manipulate very small magnetic particles.

Here, we describe the design and fabrication of a temperature-controlled electromagnetic microneedle (EMN) that is capable of applying large (1–50 nN) static or dynamic forces to micrometer- and nanometer-sized magnetic particles. Large magnetic field gradients necessary to apply force to such small particles were obtained by electrochemically sharpening the tip of the electromagnetic core to radii between 100 nm and 20 μm. Larger tip radii can be used to homogeneously apply large forces to multiple beads over large areas. Alternatively, smaller tip radii, which confine the magnetic force to within a few microns of the needle tip, may be used to selectively pull or capture single magnetic particles from within a large population of similar particles. This EMN may be useful in various applications, including the assembly of multicomponent nanometer-sized devices, molecules, and cells; measurement of molecular binding kinetics; separation of biomolecules; and micro- and nanomechanical studies of biomolecules and living cells.

The motivation for constructing the electromagnet device was to apply large (pN to nN) magnetic forces on nanometer- and micrometer-sized magnetic particles for biological applications. The magnetic force (F) on a particle depends upon the volumetric magnetization of the particle (M), the volume of the particle (V), and the gradient of the magnetic field (B) according to the equation[9,10]

$$F = V(M \cdot \nabla)B. \tag{1}$$

Even greater forces (and hence gradients) are required if these beads are bound to relatively stiff structures. For example, past studies with magnetic microbeads (4.5 μm diam-

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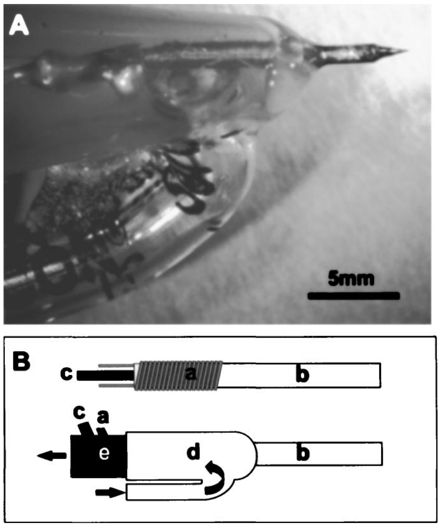

FIG. 1. *The electromagnetic needle device*. (A) Photograph of the pole tip contained within the cooling system. (B) Diagram of the internal (top) and external (bottom) views of the magnet system. A 50-μm-diameter insulated copper electromagnet wire (a) was wound around a 1-mm-diameter soft Permalloy magnet core (b). A 1.5 mm insulated copper wire (c) was soldered to the proximal end of the magnet core for electropolishing. The core with wound wire was then placed within a thermoregulating water jacket (d) fashioned from a 1.5 ml Eppendorf tube with the exposed tip of the core extending through its distal surface at the right. Arrows indicate direction of water flow through jacket and into a plastic outflow tube (e).

eter) bound to adhesion receptors on living cells revealed that forces greater than approximately 100 pN are required to produce nanometer-sized displacements.[6]

To generate the large magnetic field gradients required to move these small particles, we therefore fabricated an EMN comprised of multiple loops of insulated electromagnet wire[11] coiled around a Permalloy ™ core[12] with a pole tip that is electrochemically sharpened to submicron size (100 nm to 20 μm radius) (Fig. 1). A Permalloy rod (1 mm diameter wire) was chosen as the core material based upon its high magnetic permeability and low remnant magnetic field; its relative permeability ($\sim 1\times 10^6$) also was maximized by annealing and slow cooling in a hydrogen furnace.[12] To reduce the scale of the device for use with samples placed on a microscope platform, very fine (50 μm diameter; 44 gauge) copper wire[11] was wound around the magnetic core up to 1000 or more turns in one or more layers. A typical electromagnet composed of 2000 turns of wire had a resistance of $\sim 16\ \Omega$, an inductance of ~ 1.4 mH, and a capacitance of less than 2 pF (instrument limit). The power dropped off at higher frequencies following a relationship of -0.025 dBm/kHz out to at least 1 MHz.

The magnitude of the magnetic field gradient generated by the EMN is primarily a function of the shape of the needle tip. In order to alter and control the magnetic field gradient generated by the EMN, we electropolished a section out of the Permalloy core distal to the electromagnet coil. Importantly, the core and electromagnet wires were housed within a temperature-regulating water flow chamber [Figs. 1(a) and 1(b)] prior to the electropolishing steps to prevent heating and expansion of the device during use.

To control the sharpening of the tip of the Permalloy rod, two cylindrical plastic shields (1 mm internal diameter), cut from the ends of 200 μl Eppendorf pipette tips, were fitted over the ends of the core, leaving an exposed section of wire

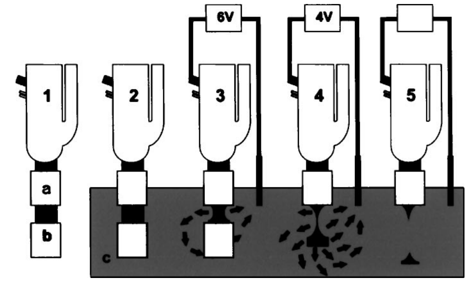

FIG. 2. *Electropolishing protocol for tailoring pole tip geometry*. (1) Two protective plastic cylindrical masks (a,b) were placed over the surface of the Permalloy core tip so that the tip was completely covered and a controllable region of the core between the masks was exposed. (2) The tip with masks was then lowered into an acid solution (c). (3) An electrical current (solid arrows) applied with a power supply set at 6 V was passed through the Permalloy core, thereby electrochemically polishing the exposed surface of the Permalloy core. Once the core had narrowed by 50%, the distal plastic cap was removed and electropolishing was continued at 4 V (4) until the distal end of the Permalloy core broke off and the current was shut down (5). The initial surface area of exposed core in step 1 determines final tip geometry.

between them (Fig. 2). The pole tip was immersed into a solution containing 8:7:5 phosphoric acid, sulfuric acid, and water, and a 6 V potential was applied to electropolish (etch) the exposed surface of the rod. When the diameter of the exposed material reached approximately 40%–50% of its original size, the potential was stopped, the distal plastic shield removed, and the electropolishing was resumed at 4 V (for better control) until the distal portion of the Permalloy tip fell off (Fig. 2). This method was highly reproducible [Fig. 3(a), inset a]. Progressively increasing the spacing between the shields from 1.5 to 15 mm resulted in a progressive increase in the taper [Fig. 3(a), insets a–d], and a decrease in the radius of the tip from approximately 6 to 0.1 μm. A pole tip with a diameter of approximately 200 nm is shown in Fig. 3(a), inset d.

Initial studies conducted using EMNs similar to the design proposed by Barbic *et al.*[9] with 20 to 80 turns of electromagnet wire and less than 2–4 Ω of resistance, but without temperature control, revealed that significant heating of the wires caused 15–20 μm movements of the needle tip when electrical currents were maintained beyond a brief pulse (>1 s; data not shown). This type of expansion and movement of the pole tip would restrict our ability to position it within short distances from magnetic particles bound to biomolecules and cells that, respectively, denature or die when heated. Movement of the magnet tip would also make it difficult to control the magnetic force gradient to which the particle is exposed as the distance between the pole tip and particle would vary over time. Thus, we deemed that an active cooling system was necessary to ensure the tip temperature remained within the design range, and to prevent the coil from melting.

Without temperature regulation, application of a current of 700 mA through an EMN containing 500 turns (4 rows of 125 turns) led to an average increase in tip temperature of 14 °C within 8 s [Fig. 3(b)]. With temperature regulation using a regulated water pump,[13] the tip temperature stabilized at approximately 2 °C above its starting value, and currents of 1 A could be maintained for extended periods of time (minutes to hours) without a significant change in temperature [Fig. 3(b)]. Importantly, the EMN tip also did not expand measurably during use.

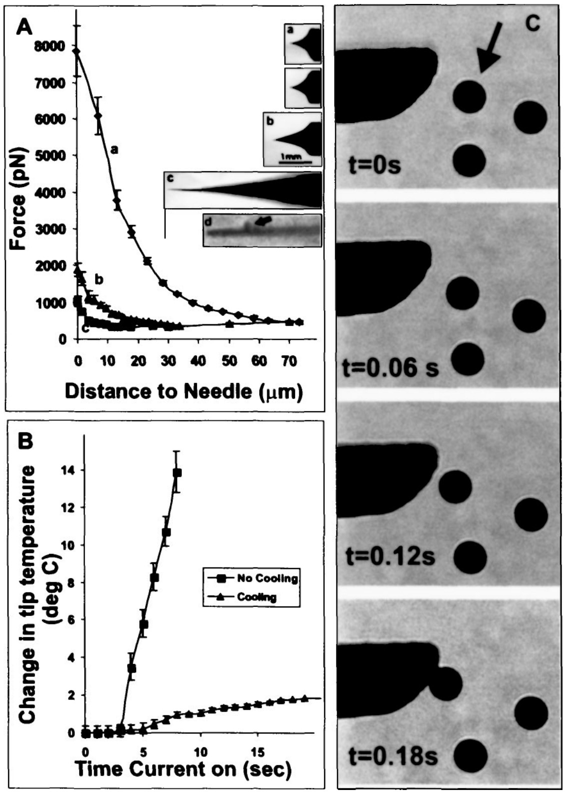

FIG. 3. (A) *Control of the magnetic field gradient by altering pole tip geometry*. EMNs, each containing 500 loops of wire, were electropolished to produce tips with different tapered shapes of increasing lengths by exposing different areas of the core using different initial separations between the two plastic masks of 1.5 (a), 3 (b), 6 (c), and 15 mm (d). In (d), a high-magnification view shows an EMN with a tip diameter of less than 200 nm (left of view); the arrow indicates a 250 nm magnetic bead bound to the side of the needle that is shown for size comparison. Repeated fabrication protocol produced similar tip geometries (a). The lines in the graph indicate the force–distance relationship for respective pole tip geometries measured using 4.5-μm magnetic beads in glycerol, as described in Ref. 5. (B) *Water-based thermoregulation of the coil prevents overheating of the magnetic needle*. Maintenance of coil current at 700 mA in a magnetic needle with 500 loops of magnet wire led to rapid overheating of needle tip (and short circuiting of the coil) within 8 s in the absence of cooling. In contrast, there was only a negligible change in temperature in the presence of water flow (15 ml/s) even when the current was maintained for extended periods of time (>45 min). (C) *Selective isolation of a single magnetic microbead from a group of closely spaced similar beads using an electropolished pole tip*. Superparamagnetic beads (4.5 μm diameter) were allowed to settle to the bottom of a glass petri dish in glycerol. A highly tapered electromagnetic microneedle created with the protocol described in 3(A), inset c was positioned nearest the bead indicated with the arrow. When a current of 100 mA was applied to the needle to create 80 pN of force on the indicated bead, the bead was pulled to the surface of the magnet. Note that because of the sharp magnetic field gradient created by this pole tip, none of the other beads moved during this process even though they were only separated by 1 to 2 bead diameters (5–10 μm).

The tractions induced by EMNs with electrochemically sharpened tips were estimated by applying forces to superparamagnetic beads (4.5 μm diameter) in a viscous glycerol solution, as described.[5] As previously observed with a stationary magnetic needle,[6] force levels increased with decreasing distance to the needle tip [Fig. 3(a)]. Lengthening the neck of the needle tip using the electropolishing technique shifted the force versus distance relationship (at 500 mA) closer to the needle tip [Fig. 3(a)]. Forces as high as 50 nN could be applied to 4.5 μm beads using an EMN with a pole tip radius of 20 μm (data not shown). Similar studies revealed that greater than 1 nN of force could be applied to 250-nm magnetic beads[14] using an EMN with a tip radius of approximately 100 nm [Fig 3(a), inset d).

Because of the steep magnetic field gradient created by the device with the longest and most highly tapered pole tip [Fig. 3(a), inset c), it was possible to use this EMN with a current of 100 mA (80 pN) to selectively pull out a single 4.5 μm magnetic bead from a group of multiple, similar magnetic beads that were separated by less than 10 μm from each other [Fig. 3(c)]. The success of this procedure confirms the sharp cutoff in the magnetic field gradient we estimated experimentally [Fig. 3(a)]. Moreover, while the separated bead is attached to the tip of the needle, it can be moved to a new location, and then released by shutting off the current.

In summary, we have designed, fabricated, and used temperature-controlled EMNs in which magnetic field gradients can be tailored by design of nanometer- and micrometer-scale core tips. This magnetic field gradient concentrator provides a versatile and relatively simple method to manipulate, probe, and position magnetic particles linked to biological molecules or living cells, when used in conjunction with an optical microscope and micromanipulator. When the pole tips are microengineered to create sharp magnetic field gradients, the EMN may be useful as a process control element within micro- or nanoassembly lines for micromanufacturing applications, including magnet-guided assembly of living cells into ordered tissue structures. By creating multiplexed arrays of similar pole tips, it also may be possible to create noninvasive magnetic switching elements for use within micro- and nanosystems, such as cellular biochip-based biosensors.

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[11]44 gauge insulated electromagnet wire available from Wire Tronic Inc, Pine Grove, California.
[12]1-mm-diameter Permalloy core wire (81% nickel, 19% iron) available from Fine Metals Corporation, Ashland, Virginia. Permalloy core annealed separately by Amuneal Manufacturing Corporation, Philadelphia, Pennsylvania.
[13]Temperature-controlled water pump (15 ml/s); Haake, West Germany.
[14]250-nm magnetic beads available from G. Kisker GbR, Germany.