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(71) Applicant (for all designated States except US): **CHILDREN'S MEDICAL CENTER CORPORATION**
[US/US]; 55 Shattuck Street, Boston, MA 02115 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **INGBER, Donald, E.** [US/US]; 71 Montgomery Street, Boston, MA 02116 (US). **MATTHEWS, Benjamin, D.** [CA/US]; 42 Adelia Avenue, West Newton, MA 02465 (US). **LAVAN, David, A.** [US/US]; ** (US). **OVERBY, Darryl** [US/US]; 22 Euston Street, Apartment 1, Brookline, MA 02446 (US).

(74) Agents: **RESNICK, David, S.** et al.; Nixon Peabody LLP, 100 Summer Street, Boston, MA 02110-2131 (US).

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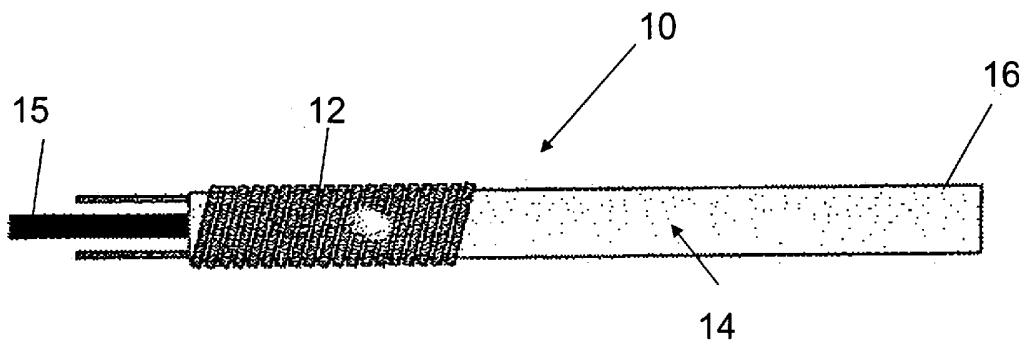
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(54) Title: APPARATUS AND METHOD FOR NANOMANIPULATION OF BIOMOLECULES AND LIVING CELLS



(57) Abstract: The present invention relates to an electromagnetic needle for magnetically manipulating microscopic particles and a method of making the same, and more particularly, to a method for nanomanipulating biomolecules and/or living cells using the electromagnetic needle of the present invention. The temperature-controlled electromagnetic microneedle includes an electromagnetic core having a magnetic probe tip. A wire coil is wound around at least a portion of the core and electrically isolated from the core. A thermo-regulating water jacket having a first and second end and enveloping the electromagnetic core and wire coil allows the probe tip to extend from the first end. The present invention also relates to a method for localized in vivo treatment of disease. Magnetically responsive particles upon which a biologically active substance is absorbed are administered to a subject. A catheter having a magnet attached thereto is inserted into the body of the subject. The magnet guides the particles to a site to be treated with the biologically active substance and causes retention of the particles at the site.

APPARATUS AND METHOD FOR NANOMANIPULATION OF BIOMOLECULES AND LIVING CELLS

GOVERNMENT SUPPORT

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RELATED APPLICATION

[0002] The present application claims benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application Serial No.: 06/614,999 filed, October 1, 2004, the contents of which are herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention:

[0003] The present invention relates to an electromagnetic needle for magnetically manipulating microscopic particles and a method of making the same, and more particularly, to a method for nanomanipulating biomolecules and/or living cells using the electromagnetic needle of the present invention.

Description of Related Art:

[0004] Occlusion of blood vessels due to blood clots is a major problem in carotid artery diseases, thrombolytic disease of various types, and all forms of interventional radiology. Due to our ever increasingly older population, this results in a huge cost to the healthcare system, and in human life. Existing approaches include delivery of clot-degrading enzymes, such as streptokinase and tissue plasminogen activator, through intravenous catheters placed at the clot site can have dramatic positive effects if delivered immediately after the clots form. However, their effectiveness is limited by the dilution of the enzyme as the clot begins to resolve and the blood washes through the opening. In addition, the total amount of enzyme that can be administered is limited by potential side effects at distant sites. Thus, if a method could be devised to deliver active enzyme only to the clot therapeutic efficacy should be increased greatly.

[0005] Magnetic micromanipulation of nanometer- and micrometer-sized magnetic particles provides a novel means to probe molecular binding interactions, *See*, J. M. Perez, L. Josephson, T. O'Loughlin, D. Hogemann, and R. Weissleder, *Nat Biotechnol* 20, 816 (2002); separate biological materials, *See*, H. Gu, P. L. Ho, K. W. Tsang, L. Wang, and B. Xu, *J Am Chem Soc* 125, 15702 (2003); characterize cell mechanical properties, *See*, N. Wang, J. P. Butler, and D. E. Ingber, *Science* 260, 1124 (1993); B. Fabry, G. N. Maksym, J. P. Butler, M. Glogauer, D. Navajas, and J. J. Fredberg, *Phys Rev Lett* 87, 148102 (2001); A. R. Bausch, F. Ziemann, A. A. Boulbitch, K. Jacobson, and E. Sackmann, *Biophys J* 75, 2038 (1998); and F. J. Alenghat, B. Fabry, K. Y. Tsai, W. H. Goldmann, and D. E. Ingber, *Biochem Biophys Res Commun* 277, 93 (2000); and control biochemical activities and gene expression within living cells, *See* C. J. Meyer, F. J. Alenghat, P. Rim, J. H. Fong, B. Fabry, and D. E. Ingber, *Nat Cell Biol* 2, 666 (2000); M. E. Chicurel, R. H. Singer, C. J. Meyer, and D. E. Ingber, *Nature* 392, 730 (1998); J. Chen, B. Fabry, E. L. Schiffrin, and N. Wang, *Am J Physiol Cell Physiol* 280, C1475 (2001). The entireties of the above-listed publications being incorporated herein by reference.

[0006] Existing magnetic manipulation techniques apply controlled mechanical stress to magnetic particles by generating either large magnetic field gradients to apply directed forces, *See* A. R. Bausch et al, *Biophys J* 75; F. J. Alenghat et al., *Biochem Biophys Res Commun* 277 and B. D. Matthews, D. R. Overby, F. J. Alenghat, J. Karavitis, Y. Numaguchi, P. G. Allen, and D. E. Ingber, *Biochem Biophys Res Commun* 313, 758 (2004), also incorporated herein by reference, or alternating magnetic field orientations to apply torques. *See*, N. Wang et al., *Science* 260.

[0007] Magnetic methods provide advantages over other techniques, such as optical tweezers, for large-scale separation of materials, manipulation of micro-particles, 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 nN level forces with magnetic techniques versus low pN forces with optical traps on micrometer-sized beads). Because of their low power requirements, miniaturized electromagnets may also be useful to non-invasively 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.

[0008] 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. *See*, M. Barbic, J.J. Mock, A.P. Gray, and S. Schultz, *Appl. Phys. Lett.* 79, 1897 (2001), incorporated herein by reference. 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.

[0009] Thus, there is a need for a temperature-controlled electromagnetic microneedle that can generate appropriate magnetic field gradients for biomedical and biophysical applications.

SUMMARY OF THE INVENTION

[0010] One aspect of the present invention is to provide a temperature-controlled electromagnetic microneedle for magnetically manipulating a magnetically responsive particle. In one embodiment, the microneedle is formed from an electromagnetic core having a magnetic probe tip. For certain applications, the core is a soft magnetic wire having a diameter of 1 mm or less. A wire is wound around at least a portion of the core forming a coil. Preferably, the wire is copper and has a diameter of 50 μm or less. The wire is electrically isolated from the core. The needle further has a thermo-regulating water jacket enveloping the electromagnetic core and wire coil but allowing the probe tip to extend from the bottom portion. A current source for supplying current to the coil is also included.

[0011] As is discussed in more detail below, the probe tip is preferably formed by electrochemically etching the end of the electromagnetic core. Preferably, the tip has a diameter between 5 nm and 5 mm, more preferably, between 200 nm and 20 μm .

[0012] Another aspect of the present invention is to provide a method of forming an electromagnetic pole tip. The method includes providing an electromagnetic core and a wire coil wound around at least a portion of the core and electrically isolated from the core or a stationary magnetic core. The core is preferably permalloy. A proximal and distal protective mask are attached over the portion of the surface of the core not having the wire coil wound around. The distal mask covers the end portion of the core and the proximal mask is attached at a sufficient distance from the distal mask to provide for an exposed region of the core between the masks. The core is then immersed in an acid solution to a depth not exceeding the proximal mask. Preferably, the acid is a mixture of phosphoric and sulfuric acids. An electrical current is applied through the core for a time period sufficient to allow the exposed region to erode by about a desired amount, preferably about 40-50%. The distal mask is then removed and a second electrical current is applied for a time period sufficient to allow the core previously under the distal mask to detach, resulting in the pole tip.

[0013] Preferably, for added control of the process, the second electrical current is lower than the first. The process allows one to increase the taper of the pole tip and decreases the radius of the tip by increasing the distance between the proximal and distal masks.

[0014] Still another aspect of the present invention is a catheter including an electromagnetic pole tip produced by the method of the present invention.

[0015] In a further aspect of the present invention, a plurality of electromagnetic pole tips of the invention are used to form multiplexed arrays.

[0016] Yet another aspect of the present invention is a method for localized in vivo treatment of disease. In the method, magnetically responsive particles having a biologically active substance selected for its efficacy in treating the disease, absorbed on the particles, are administered to the patient being treated. The particles can be administered by any means, including, for example, a catheter, systemic I.V. injection or surgical incision. At a time thereafter, before or simultaneously therewith, a catheter having a magnet attached thereto is inserted in the body being treated. The magnet can be an electromagnet, including an electromagnet of the invention, or a stationary magnet. The magnet is then contacted with the site to be treated. The

magnet has sufficient strength to guide a substantial quantity of the particles to, and retain the substantial quantity of the particles at the site. In a preferred embodiment, the particles are administered through the catheter. In another embodiment, a substantial quantity of the particles is removed from the body through the catheter.

[0017] The site can be any location capable of being treated with a biologically active agent. For example, if the site to be treated is a blood clot, the biologically active substance is a clot degrading compound, for example, streptokinase or tissue plasminogen activator. Alternatively, if the site to be treated is a hemorrhage, the biologically active substance is a clot-promoting compound, for example, thrombin or tissue transglutaminase. In another embodiment, when the site to be treated is tumor vasculature, the biologically active substance is a clot-promoting compound. In still another embodiment, the site to be treated is an aneurysm, e.g. a vascular aneurysm, or a venous malformation and the biologically active substance is a clot-promoting compound.

[0018] In order to control the activity of certain biologically active substance, e.g., enzymes, inactive fragments are absorbed on different particles. When the particles are brought into close proximity, enzymatic activity is restored. Thus, if the particles should travel from their intended site, the substance will have little or no adverse effect.

[0019] In one embodiment of the present invention, the particles and/or catheter is coated with a visualization tag. The particles can further include a targeting molecule that directs and concentrates the particles at the site to be treated. For example, particles used in the treatment of hemorrhage can contain a collagen targeting molecule. For the treatment of tumor vasculature, the particle can include an angiogenesis targeting molecule such as an antibody or binding molecule directed against an integrin.

[0020] For the intravascular methods of the present invention, the particle diameter preferably ranges from 20nm to 2 micrometers. For non-intravascular methods, can be larger, up to 5 millimeters in diameter.

[0021] These and other features, aspects, and advantages of the present invention will become more apparent from the following detailed description of the preferred embodiment relative to the accompanied drawings, in which:

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Fig. 1 is a top, internal view of the EMN device of the present invention.

[0023] Fig. 2 is an external bottom view of the EMN device enclosed within the cooling system.

[0024] Fig. 3, is a photograph of the pole tip contained within the cooling system.

[0025] Fig. 4 is a perspective view of the system used for forming the EMN device of the present invention.

[0026] Fig. 5A is a graph of the force of the magnetic field gradient and the tip geometry and Fig. 5B is photographs of numerous tips and particles.

[0027] Fig. 6 is a graph comparing the change in Tip temperature and the time the current is applied.

[0028] Fig. 7 is a block illustration of the various components of the EMN system of the present invention.

[0029] Fig. 8 is a perspective view of a catheter having an electromagnetic pole tip of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0030] The present invention provides 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 may be obtained by electrochemically sharpening the tip of the electromagnetic core to diameters between 200 nm and 20 μ m. In one embodiment, larger tip radii can be used to homogenously apply large forces to multiple beads over large areas. In an

alternative embodiment, smaller tip radii (0.2 – 6 μm), 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. The EMN of the present invention is useful in various applications, including the assembly of multi-component nanometer sized devices, molecules and cells; measurement of molecular binding kinetics; separation of biomolecules; and micro- and nano-mechanical studies of biomolecules and living cells.

[0031] In a preferred embodiment, the electromagnet device is used 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:

$$F = V(M \cdot \nabla) B \quad (1)$$

See, M. Barbic, J.J. Mock, A.P. Gray, and S. Schultz, *Appl. Phys. Lett.* 79, 1897 (2001) and Q. A. Pankhurst, J. Connolly, S. K. Jones, and J. Dobson, *J Phys D: Appl Phys* 36, R167 (2003), incorporated herein by reference.

[0032] 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 diameter) bound to adhesion receptors on living cells revealed that forces greater than approximately 100 pN are required to produce nanometer-sized displacements.

[0033] Referring to Fig. 1, to generate the large magnetic field gradients required to move such small particles, an EMN 10 having multiple loops of insulated electromagnet wire 12, for example, a 50 μm diameter or less, 44-gauge insulated electromagnet copper wire, available from WireTronic Inc, Pine Grove, California, coiled around a soft permalloy magnetic core 14 having a diameter of about 1 mm or less. It should be appreciated that wire 12 is electrically isolated from core 14 and can be wrapped around at least a portion of core 14. A pole tip 16 extends from core 14. A, for example, 1.5 mm insulated copper wire 15 is soldered to the proximal end of the magnetic core 14 for electropolishing. As will be described further herein, tip 16 can be electropolished, that is electrochemically sharpened to a submicron

diameter of 5 nm and 5 mm, or more preferably, 200 nm to 20 μ m diameter. Permalloy rod 14 can be chosen from a material having a high magnetic permeability and low remnant magnetic field, for example, a 1 mm diameter permalloy core wire (81% Nickel 19% Iron) available from Fine Metals Corporation, Ashland, Virginia. It should be appreciated that other materials and diameters of the core and wires are contemplated by the present invention.

[0034] The relative permeability, for example, $\sim 1 \times 10^6$, can also be maximized by annealing and slow cooling in a hydrogen furnace, for example, the permalloy core can be annealed separately by Amuneal Manufacturing Corporation, Philadelphia, Pennsylvania.

[0035] Referring to Figs. 2 and 3, the cooling system of the present invention includes core 14 with wound wire 12 disposed within a thermo-regulating water jacket 20, for example a 1.5 ml Eppendorf tube. The exposed tip 16 of core 14 extends through a distal surface of cooling jacket 20. Pole tip 16 thus extends from one end of the jacket. Jacket 20 can completely envelope wire wrapped core 14 or just a part thereof. During operation, water flows through jacket 20 in the direction of the arrows indicated in Fig. 2 and into an outflow tube 18 located at an end thereof to cool the device as described herein. Outflow tube 18 can be made of plastic or any other suitable material.

[0036] Fig. 4 illustrates the electropolishing protocol for tailoring the geometry of pole tip 16. As described supra, probe tip 16 is formed by electrochemical etching. As shown in step (a) of Fig. 4, two protective plastic cylindrical masks, proximal protective mask 22 and distal protective mask 24 are positioned over the surface of the permalloy core tip 16. Distal mask 24 covers the end portion of the core and proximal mask 22 is attached to the core at a sufficient distance from distal mask 24 to provide for an exposed region 26 therebetween. Thus, tip 16 is completely covered and controllable region 26 of the core between the masks is exposed. Next, illustrated by step (b), the tip with masks 22, 24 is lowered into an acid solution 30 to a depth not exceeding the proximal mask. A first electrical current indicated by solid arrows 28 in step (c) is applied with a power supply 32 set at, for example, 6V is passed through the permalloy core, for a desired time thereby electrochemically polishing the exposed surface 26 of the permalloy core. Once the

core had narrowed by approximately 40-50%, the distal plastic mask 24 is removed, see step (d) and a second electrical current, for example, 4V, is applied for an additional time period until the distal end of the permalloy core breaks off or detaches and the current is shut down, see step (e). It should be appreciated that the amount of core exposed in region 26 is variable and the amount of energy applied thereto is variable. However, the initial surface region 26 of the core in exposed step (a) determines the final tip geometry.

[0037] The second electrical current applied to the exposed region 26 should be lower than the first electrical current. Moreover, it should be appreciated that increasing the exposed region 26, i.e., increasing the distance between masks 22, 24 increases the taper of pole tip 16 and decreases the radius of the tip.

[0038] Figs. 5A and 5B illustrate results of experiments fully described further herein and demonstrate the control of the magnetic field gradient by altering the pole tip geometry. A plurality of EMNs, each containing 500 loops of wire, were electropolished to produce tips having different tapered shapes of increasing lengths by exposing different areas of the core using different initial separations between the two plastic masks of (a) 1.5 mm, (b) 3 mm, (c) 6 mm and (d) 15 mm. 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 the graph indicate the force-distance relationship for respective pole tip geometries measured using 4.5 μ m magnetic beads in glycerol, as described by F. J. Alenghat, *Biochem Biophys Res Commun* 277.

[0039] In Fig. 6, water-based thermoregulation of the coil prevented overheating of the magnetic needle. Maintenance of a coil current at 700 mA and a magnetic needle with 500 loops of magnet wire led to rapid overheating of needle tip (and short circuiting of the coil) within 12 secs in the absence of cooling. In contrast, there was only a negligible change in temperature in the presence of water flow (15 ml/sec) even when the current was maintained for extended periods of time (>45 min). Referring back to Fig. 5B (c) the selected isolation of a single magnetic microbead from a group of closely spaced similar beads using an electropolished pole tip occurred. Superparamagnetic beads (4.5 μ m 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 Fig 5A, 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).

[0040] 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 was wound around the magnetic core up to 1000 or more turns. The higher the number of turns, the more heat is generated. As more heat is generated, a greater flow of water over the coil for temperature is required to regulate the temperature of the tip in one or more layers.

[0041] Up to 4 layers were tested. Cooling efficiency decreased with the increasing number of layers. A typical electromagnet composed of 2000 turns of wire had a resistance of ~ 16 ohms, an inductance of $\sim 1.4\text{mH}$, 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 1MHz. The magnitude of the magnetic field gradient generated by the EMN was primarily a function of the shape of the needle tip. In order to alter and control the magnetic field gradient generated by the EMN, a section out of the permalloy core distal to the electromagnet coil was electropolished. Importantly, the core and electromagnet wires were housed within a temperature-regulating water flow chamber (Fig. 2) prior to the electropolishing steps to prevent heating and expansion of the device during use.

[0042] 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 between them (Fig. 4). The pole tip was immersed into a solution containing 8:7:5 phosphoric acid, sulfuric acid, and water, and a 6V 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 4V (for better control) until the distal portion of the permalloy tip fell off (Fig 2). This method was highly reproducible (Fig. 5A, inset a). Progressively increasing the spacing between the shields from 1.5 to 15 mm resulted in a progressive increase in the taper (Fig. 4A, insets a-d), and decrease in the radius of the tip from approximately 6 to 0.2 μm . A pole tip with a diameter of approximately 200 nm is shown in Fig. 4A, inset d.

[0043] Initial studies conducted using an EMN similar to the design proposed by Barbic et al., with 20 to 80 turns of electromagnet wire and less than 2-4 ohms 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 sec; data not shown). This type of expansion and movement of the pole tip restricts the user's 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 also makes 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. These problems were overcome by use of an active cooling system, which ensures the tip temperature remained within the design range, and to prevent the coil from melting.

[0044] 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 12 secs (Fig. 3B). With temperature regulation using a regulated water pump, for example, a temperature controlled water pump having a flow rate of 15 ml/sec. manufactured by Haake of West Germany could be used, the tip temperature stabilized at approximately 2°C above its starting value, and currents of 1A could be maintained for extended periods of time (minutes to hours) without a significant change in temperature (Fig. 4B). Importantly, the EMN tip also did not measurably expand during use.

[0045] 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 by F. J. Alenghat et al.. As previously observed with a stationary magnetic needle, see B.D. Matthews et al., force levels increased with decreasing distance to the needle tip (Fig. 5A). 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. 5A). 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 . Similar studies revealed that greater than 1 nN of force could be applied to 250 nm magnetic beads, for example, 250 nm beads available from G. Kisker gbR, Germany, using an EMN with a tip radius of approximately 200 nm (Fig 5B, inset d).

[0046] Because of the steep magnetic field gradient created by the device with the longest and most highly tapered pole tip (Fig. 5A, 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. 5B (c)). The success of this procedure confirms the sharp cutoff in the magnetic field gradient estimated experimentally (Fig. 5A). 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. Further, using the device of the present invention, removal of a single 4.5 μm superparamagnetic bead from a group of similar ones, less than 10 μm from each other.

[0047] Fig. 7 is a block diagram illustrating the various components of the EMN system of the present invention. EMN device 10 is connected to insulated wires 46 that supply electrical current to the electromagnet created by the device. Tubing 42 supplies inflow of cooling water to device 10. Outflow tubing 44 returns the fluid from the device. A temperature regulated water pump 36 completes the loop of cooling flow. The cooling system prevents the coil of the device from overheating.

[0048] To control the positioning of the device a micromanipulator 48 is provided. Micromanipulator 48, for example, an Eppendorf manipulator, can be controlled by a joystick, not shown. A screen of a microprocessor 50 allows the user to monitor the positioning of the device. In use, a sample of cells and particles located, for example, in a Petri dish are exposed to the EMN device. A microscope 38 magnifies the cells and particles to observe the above phenomena when the EMN device is used.

[0049] As shown in Fig. 8, a catheter 60 includes an electromagnetic pole tip 62 produced by the method of the present invention. Catheter 60 can include a visualization tag 64. Catheter 60 can be used as part of a method for localized in vivo

treatment of disease providing magnetically responsive particles having a biologically active substance selected for its efficacy in treating the disease absorbed on said particles. Catheter 60 can be inserted into a body being treated. Magnetically responsive particles having a biologically active substance selected for its efficacy in treating the disease, absorbed on the particles, are administered to the patient being treated. The particles can be administered by any means, including, for example, a catheter, systemic I.V. injection or surgical incision. At a time thereafter, before or simultaneously therewith, the catheter having a magnet attached thereto is inserted in the body being treated. Magnet 62 can be an electromagnet, including an electromagnet of the invention, or a stationary magnet. The magnet is then contacted with the site to be treated. The magnet has sufficient strength to guide a substantial quantity of the particles to, and retain the substantial quantity of the particles at the site. In a preferred embodiment, the particles are administered through the catheter. In another embodiment, a substantial quantity of the particles is removed from the body through the catheter.

[0050] 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 nano-assembly 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 non-invasive magnetic switching elements for use within micro- and nano-systems, such as cellular biochip-based biosensors.

[0051] In the field of clot removal, the above effect can be enhanced by being able to position the enzyme activity precisely where desired and to visualize the position of the enzyme as well as the response to therapy in real-time. In addition, the ability to remove the enzyme after the occlusion has been opened, and to deliver enzyme that immediately loses its catalytic activity if it travels more than a few millimeters from the focus site could provide a means to deliver greatly increased amounts of enzyme to the clot. This would greatly accelerate the process of clot removal and hence minimize morbidity and mortality.

[0052] Enzymes, such as streptokinase and TPA, can be held in place at the clot site by being immobilized on the surfaces of nanometer-sized magnetic particles

if a stationary magnet or electromagnet is placed at the tip of the intravenous catheter that also delivers the magnetic particles. The particles may be delivered through a second port upstream if there is a stationary magnet at the catheter tip which is in direct contact with the clot. The presence of the magnet would hold the bead-enzyme complexes at the clot site even after blood started to flow through the first opening in the occlusion. The magnetic particles can be visualized using MRI and possibly by other imaging modalities, especially if they are also coated with different types of visualization tags. Once the clot is fully removed, the catheter and bound cluster of nanoparticles/enzymes can be removed through the catheter. Because the particles can be iron oxide-based materials already approved by the FDA as MRI contrast reagents, it is known that any remaining particles that are sheared off the tip of the bead will be cleared by the liver and passed out of the body through the bile.

[0053] In another embodiment, the magnetic particles are released free and allowed to interact with tissue sites. Thereafter, the magnet is applied to remove or reposition the particles. Alternatively, magnetic particles are placed on the tip of the magnetic catheter and used to direct the particles to the site. In this scenario, the particles might not be introduced i.v.; rather they could be placed on the tip of the catheter 60 before it is introduced so that they are not free in the circulation (for example). Then once at the site, the magnet could be pulled back from tip (or current turned off) and the particles could be released free. This embodiment might better concentrate the particles in one localized site and minimize occlusion, side effects, etc.

[0054] By “magnetically responsive particles” as used herein, the phrase is intended to include any microscopic bead that is capable of being administered *in vivo*. Beads suitable for use as a starting material and in accordance with the present invention are generally known in the art and can be obtained from manufacturers such as Spherotech Inc., Advanced Magnetix Inc., Kisker, Miltenyl-Biotec, Dynal Biotech, and Ferro Tec USA. See, www.magneticmicrosphere.com/supply.htm. Preferred are 20 – 0150 nm nanoparticles from Advanced Magnetix, Inc. (FERIDEX, COMBIDEX). See, WO 94/16683; 6,274,121 for exemplary particles. Suitable particles for intravascular application have an average diameter of about 20 nm to 2 μ m. A biologically active substance is absorbed onto the particles. The substance, e.g., a small molecule, protein or peptide, can be absorbed or attached to the particle

using standard methods in the art including, for example, conventional linker chemistry including tosyl-activated groups, carbodiimide chemistry, etc.

[0055] Biologically active substrates included, for example, antitumor protein, enzyme, antitumor enzyme, antibiotic, plant alkaloid, alkylation reagent, antimetabolite, hormone and hormone antagonist, interleukin, interferon, growth factor, tumor necrosis factor, endotoxin, lymphotoxin, urokinase, streptokinase, plasminogen-streptokinase activator complex, tissue plasminogen activator, macrophagen activating body antisera, protease inhibitor, substance containing radioactive isotope, cardiovascular pharmaceutical agents, chemotherapeutics, gastrointestinal pharmaceutical agents, neuropharmaceutic agents, angiogenesis inhibitors, antibodies, peptides, aptamers, catalytic entities or enzymes that cleave inactive pro-forms of molecules or drugs into their active forms.

[0056] In summary, the designed, fabricated and used temperature-controlled EMNs of the present invention, have magnetic field gradients that can be tailored by design of nanometer- and micrometer-scale core tips. This novel 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.

[0057] Although the present invention has been described in relation to particular embodiments thereof, many other variations and modifications and other uses will become apparent to those skilled in the art. It is preferred therefore, that the present invention be limited not by the specific disclosure herein, but only by the appended claims.

[0058] The references cited throughout the specification are incorporated herein in their entirety.

CLAIMS

We claim:

1. A temperature-controlled electromagnetic microneedle for magnetically manipulating at least one microscopic particle comprising:
 - a. an electromagnetic core having a magnetic probe tip;
 - b. a wire coil wound around at least a portion of said core and electrically isolated from said core;
 - c. a thermo-regulating water jacket having a first and second end and enveloping the electromagnetic core and wire coil but allowing said probe tip to extend from the first end.
2. The microneedle of claim 1, wherein the probe tip is formed by electrochemically etching the end of the electromagnetic core.
3. The microneedle of claim 1, wherein the tip has a diameter between 5 nm and 5 mm.
4. The microneedle of claim 1, wherein the tip has a diameter between 200 nm and 20 μ m.
5. The microneedle of claim 1, further comprising: a current source for supplying current to said coil.
6. The microneedle of claim 1, wherein said electromagnetic core has a diameter of 1mm or less.
7. The microneedle of claim 1, wherein said wire coil comprises wire having a diameter of 50 μ m or less.
8. A catheter comprising the microneedle of claims 1-7.

9. A method for forming an electromagnetic or stationary magnet pole tip comprising the steps of:
 - a. providing an electromagnetic core and a wire coil wound around at least a portion of the core and electrically isolated from the core or a magnetic core;
 - b. attaching a proximal and distal protective mask over the portion of the surface of the core not having the wire coil wound around, wherein the distal mask covers the end portion of the core and the proximal mask is attached at a sufficient distance from the distal mask to provide for an exposed region of the core between the masks;
 - c. immersing the masked core in an acid solution to a depth not exceeding the proximal mask;
 - d. applying a first electrical current through the core for a time period sufficient to allow the exposed region to erode by about a desired amount;
 - e. removing the distal mask; and
 - f. applying a second electrical current for a time period sufficient to allow the core previously under the distal mask to detach, resulting in a pole tip.
10. The method of claim 9, wherein the second electrical current is lower than the first.
11. The method of claim 9, wherein increasing the distance between the proximal and distal masks increases the taper of the pole tip and decreases the radius of the tip.
12. The method of claim 9, wherein the tip has a diameter of between about 50 nm and 5 millimeters.
13. The method of claim 9, wherein the desired amount is 40-50%.

14. A method for localized in vivo treatment of disease comprising:
 - a. providing magnetically responsive particles having a biologically active substance selected for its efficacy in treating the disease absorbed on said particles;
 - b. inserting a catheter in a body being treated, said catheter having a magnet attached thereto;
 - c. administering said particles to said body;
 - d. contacting said magnet to a site to be treated, said magnet having sufficient strength to guide a substantial quantity of said particles to or in close proximity to said site, and retain said substantial quantity of said particles at said site or in close proximity to said site.
15. The method of claim 14, wherein said particles are administered by said catheter.
16. The method of claim 14, further comprising removing a substantial quantity of said particles from said body by said catheter.
17. The method of claim 14, wherein said site to be treated is a blood clot and said biologically active substance is a clot degrading compound.
18. The method of claim 17, wherein the clot degrading compound is streptokinase or tissue plasminogen activator.
19. The method of claim 14, wherein said particles are coated with a visualization tag.
20. The method of claim 14, wherein said catheter is coated with a visualization tag.
21. The method of claim 14, wherein fragments of the biologically active substances are absorbed on different particles and preferentially exhibit enzymatic activity when brought into close proximity.

22. The method of claim 14, wherein the site to be treated is a hemorrhage and said biologically active substance is a clot-promoting compound.
23. The method of claim 22, wherein the clot promoting compound is thrombin or tissue transglutaminase.
24. The method of claim 14, wherein the particles further comprises targeting molecules that direct and concentrate the particles at the site to be treated.
25. The method of claim 22, further comprising a collagen targeting molecule.
26. The method of claim 14, wherein the site to be treated is tumor vasculature and said biologically active substance is a clot-promoting compound.
27. The method of claim 26, further comprising an angiogenesis targeting molecule.
28. The method of claim 27, wherein the angiogenesis targeting molecule is an antibody or binding molecule directed against an integrin.
29. The method of claim 14, wherein the biologically active substance is an enzyme that activates a prodrug.
30. The method of claim 14, wherein the magnet is an electromagnet.
31. The method of claim 14, wherein the magnet is a stationary magnet.
32. The method of claim 14, wherein the treatment involves an intravascular application and the particle diameter ranges from 2 nm to 2 micrometers.
33. The method of claim 14, wherein the treatment is non-intravascular and the particle diameter ranges from 200 nm to 5 mm.

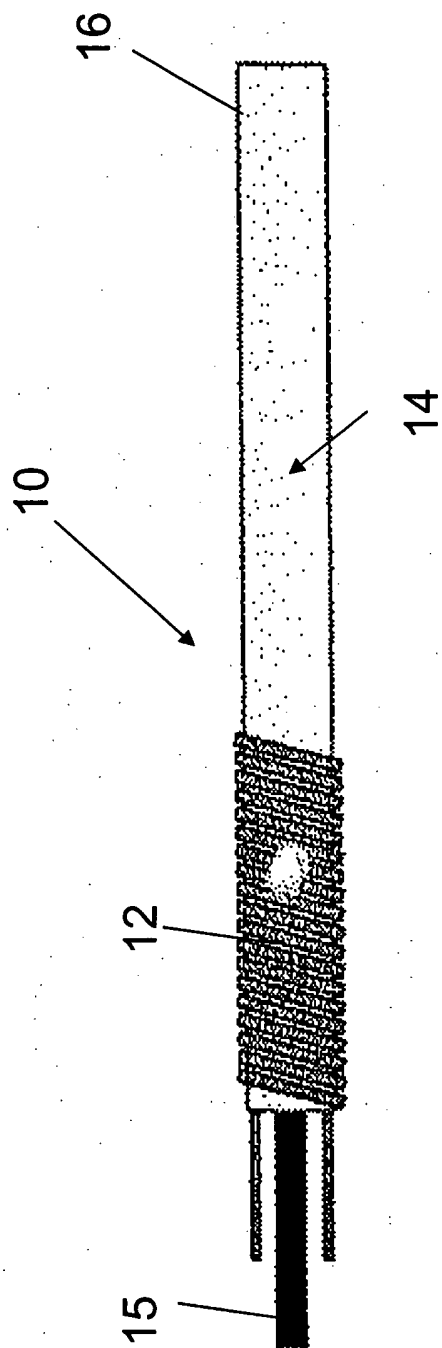


Fig. 1

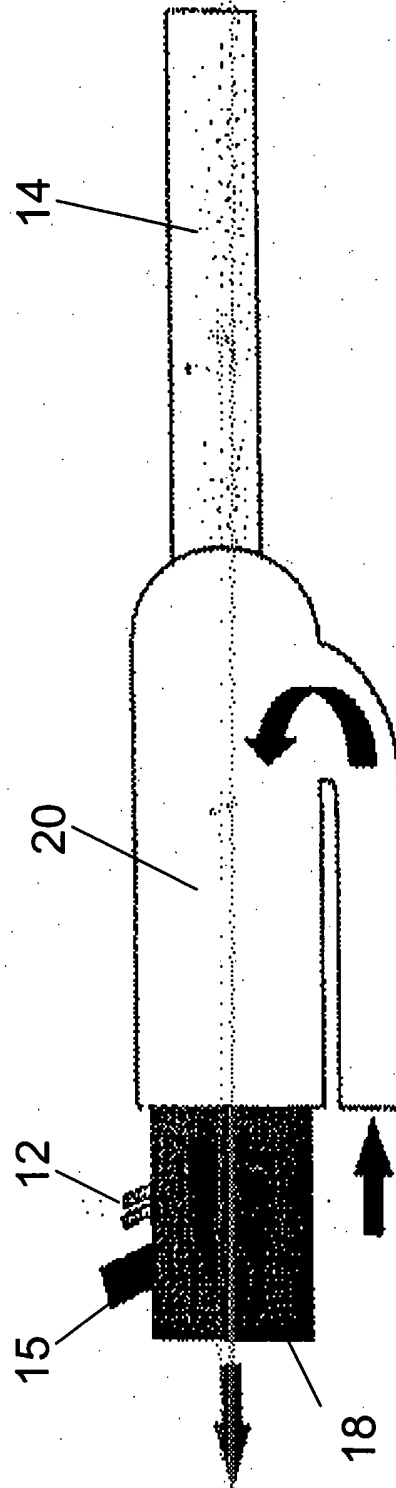


Fig. 2

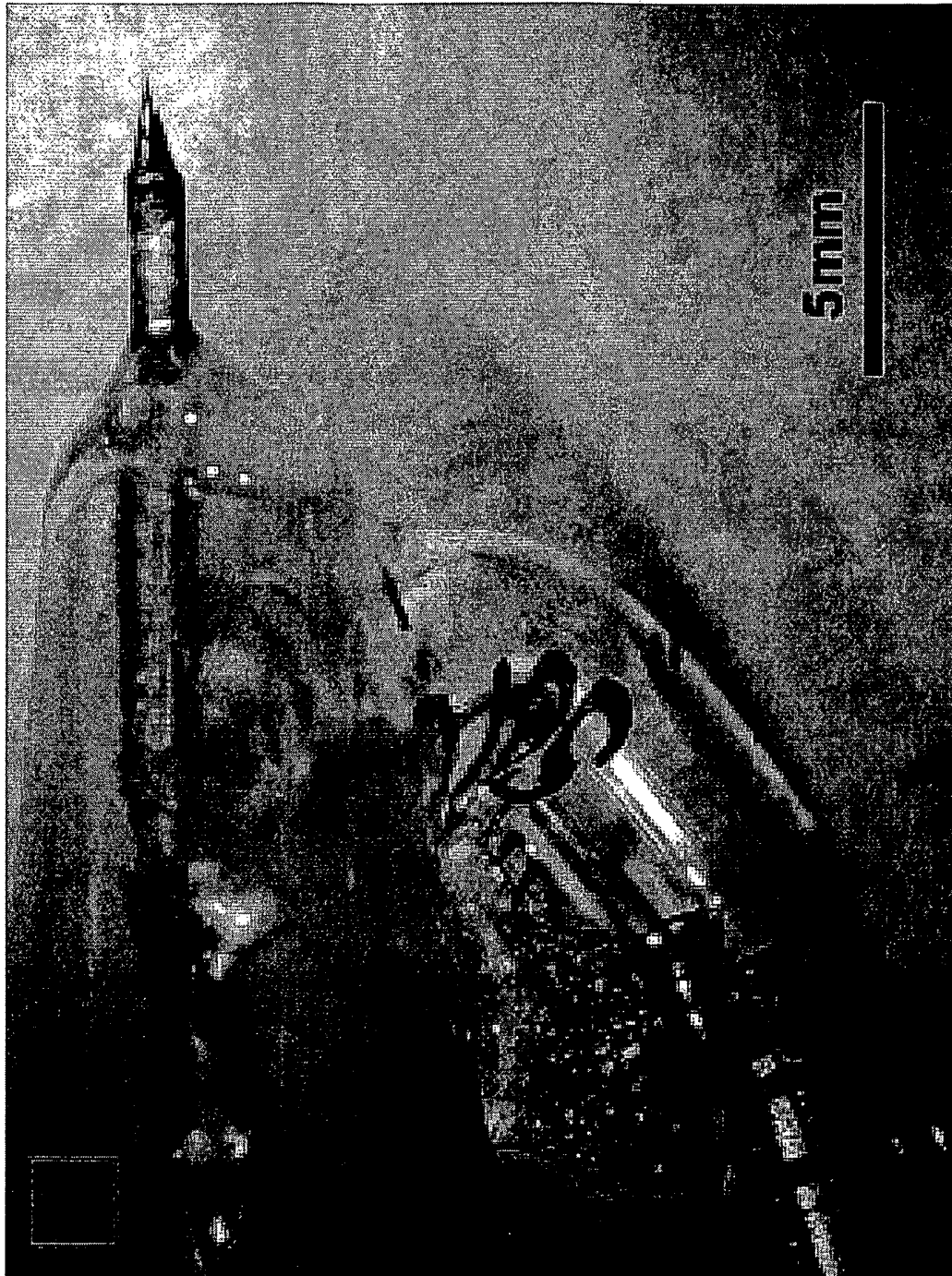
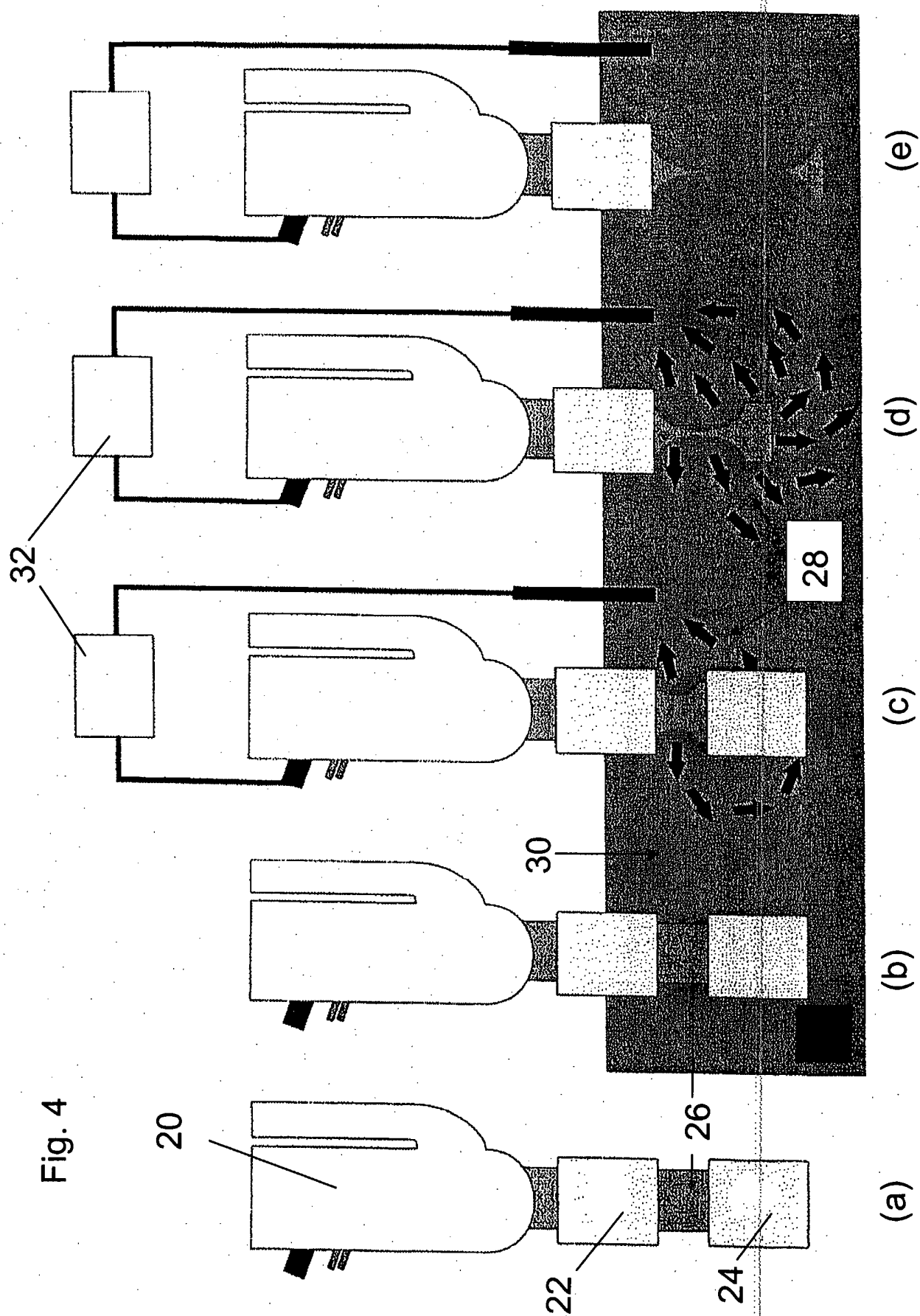


Fig. 3



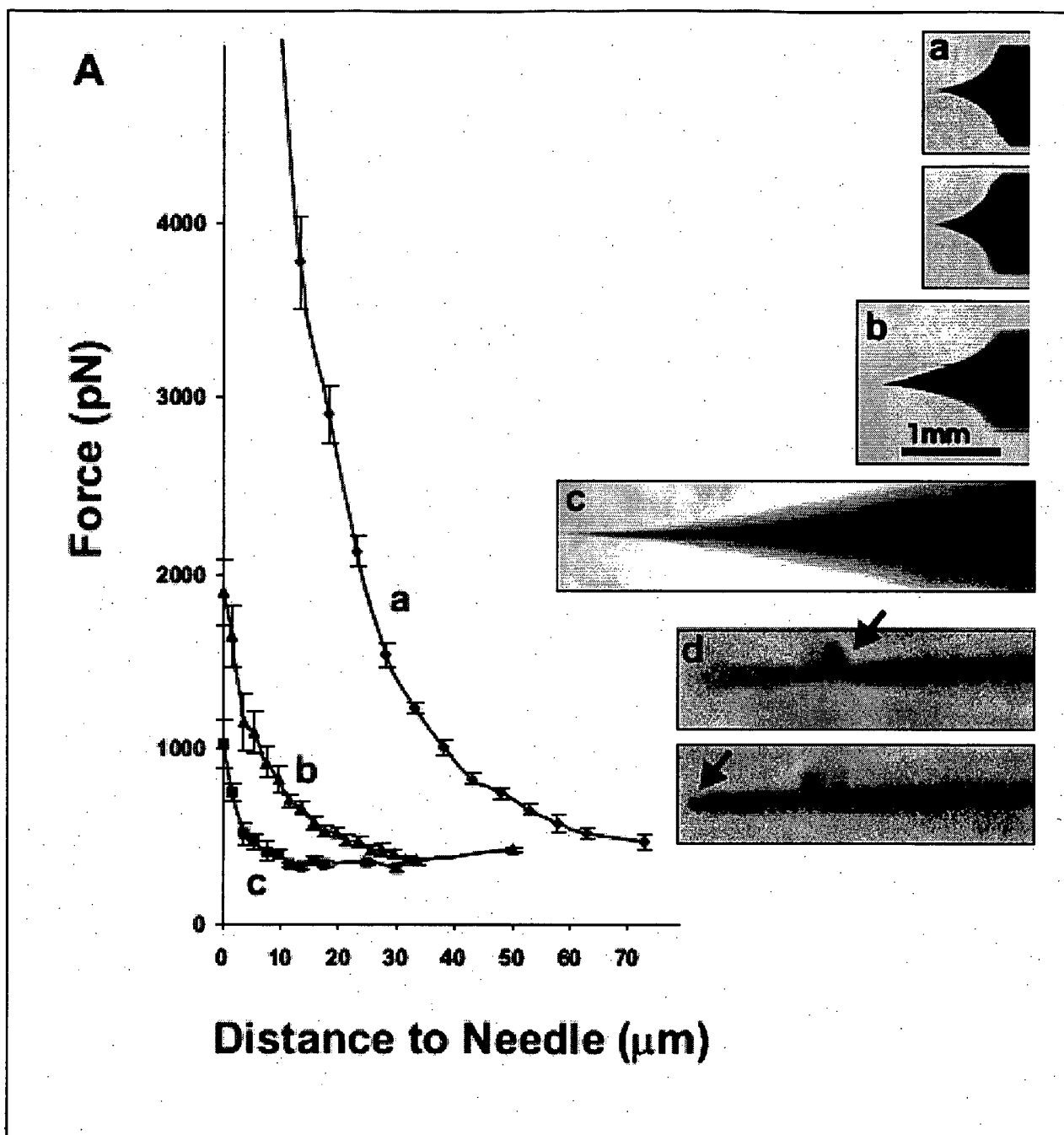


Fig. 5A

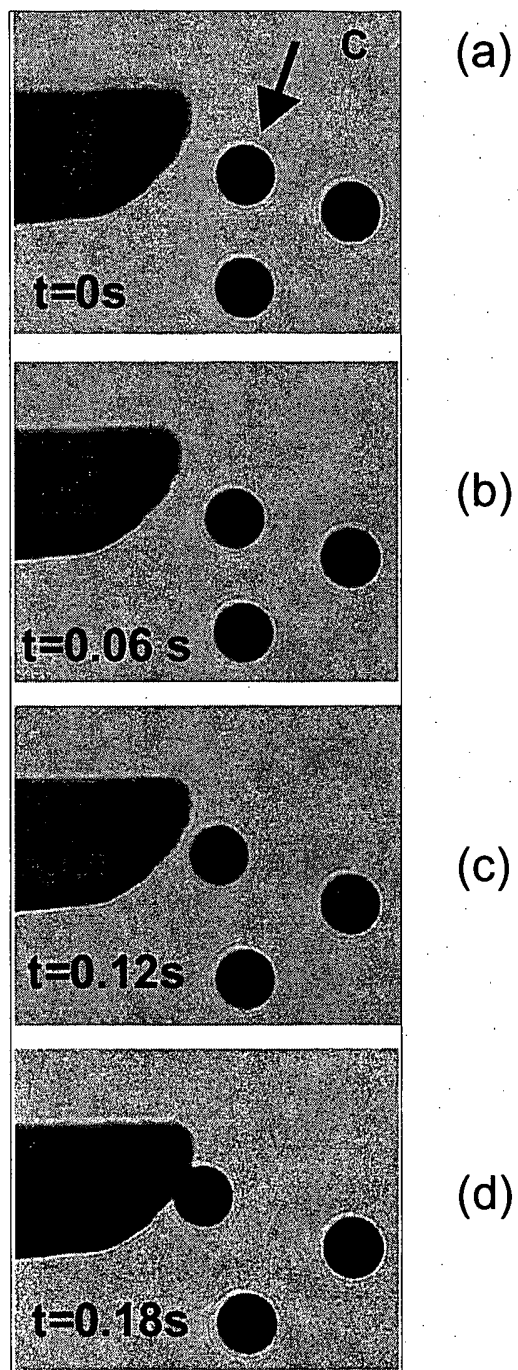


Fig. 5B

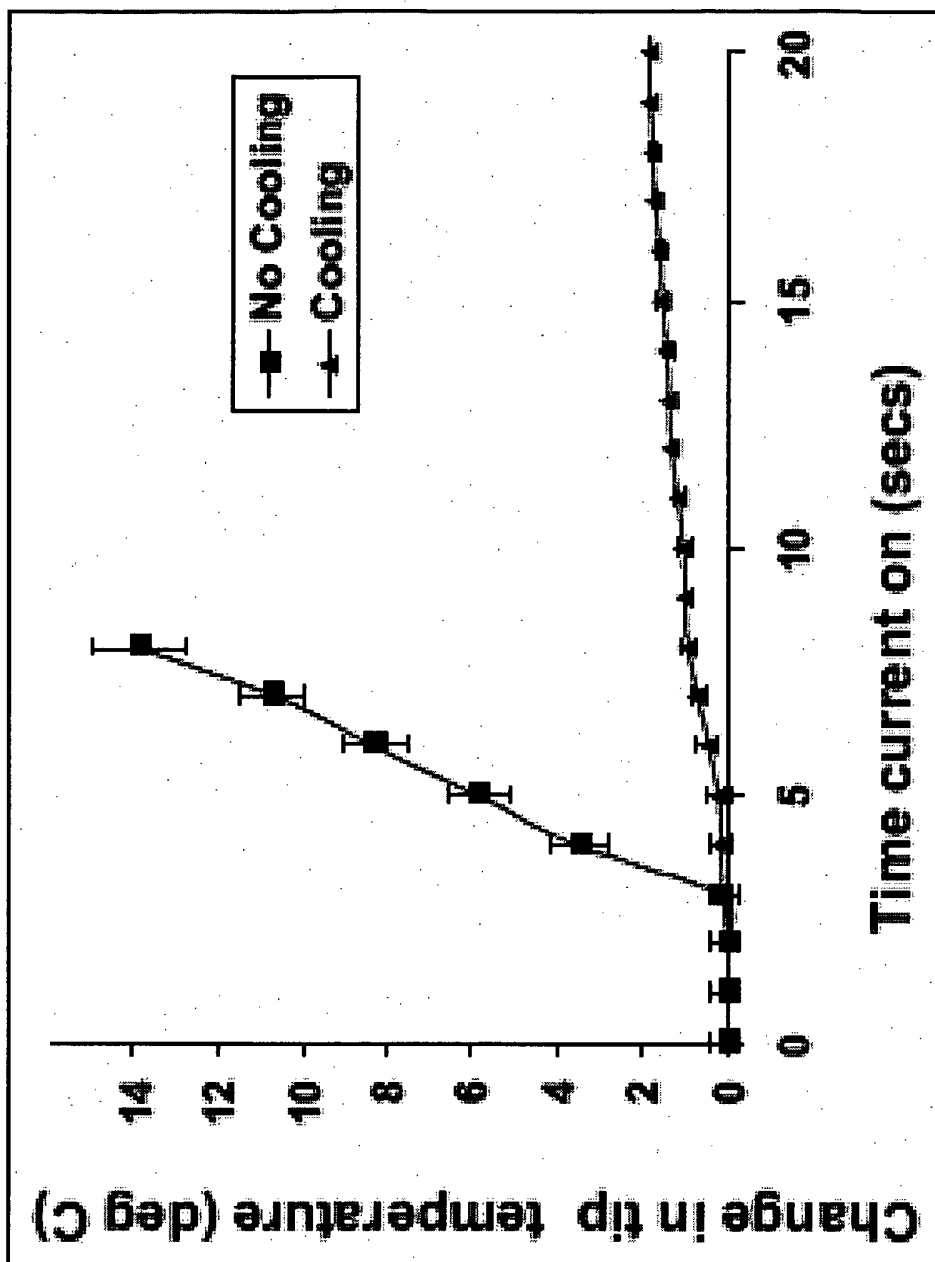
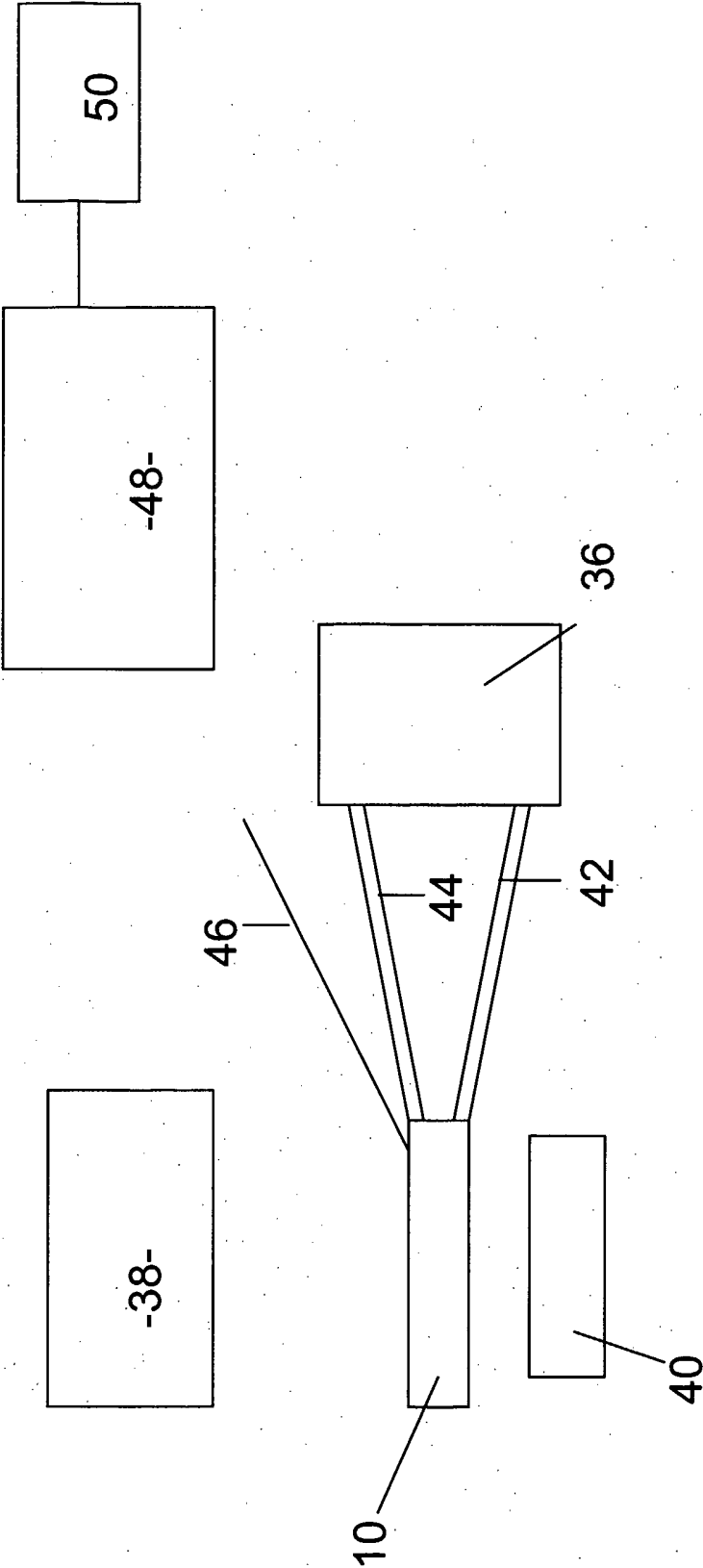


Fig. 6

Fig. 7



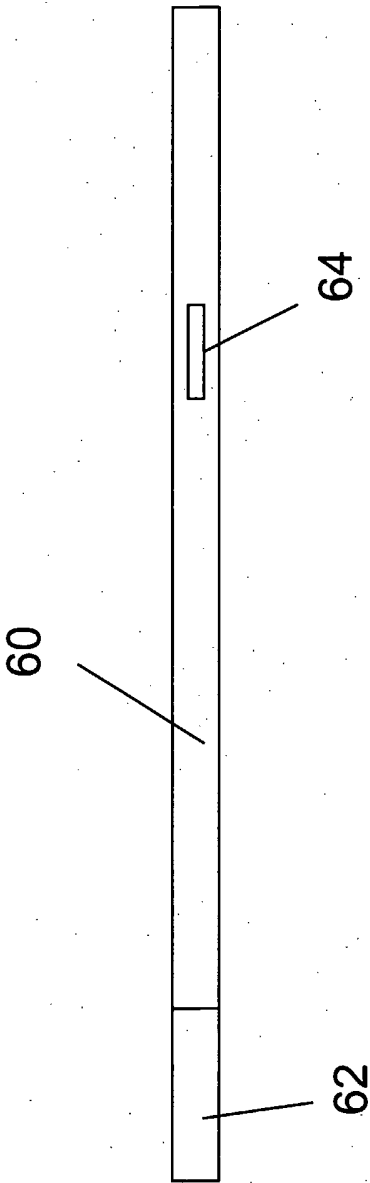


Fig. 8