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(19) **United States**(12) **Patent Application Publication**
Marchitto et al.(10) **Pub. No.: US 2004/0129555 A1**(43) **Pub. Date: Jul. 8, 2004**(54) **ENERGY ENHANCED REACTION
CATALYSIS AND USES THEREOF**

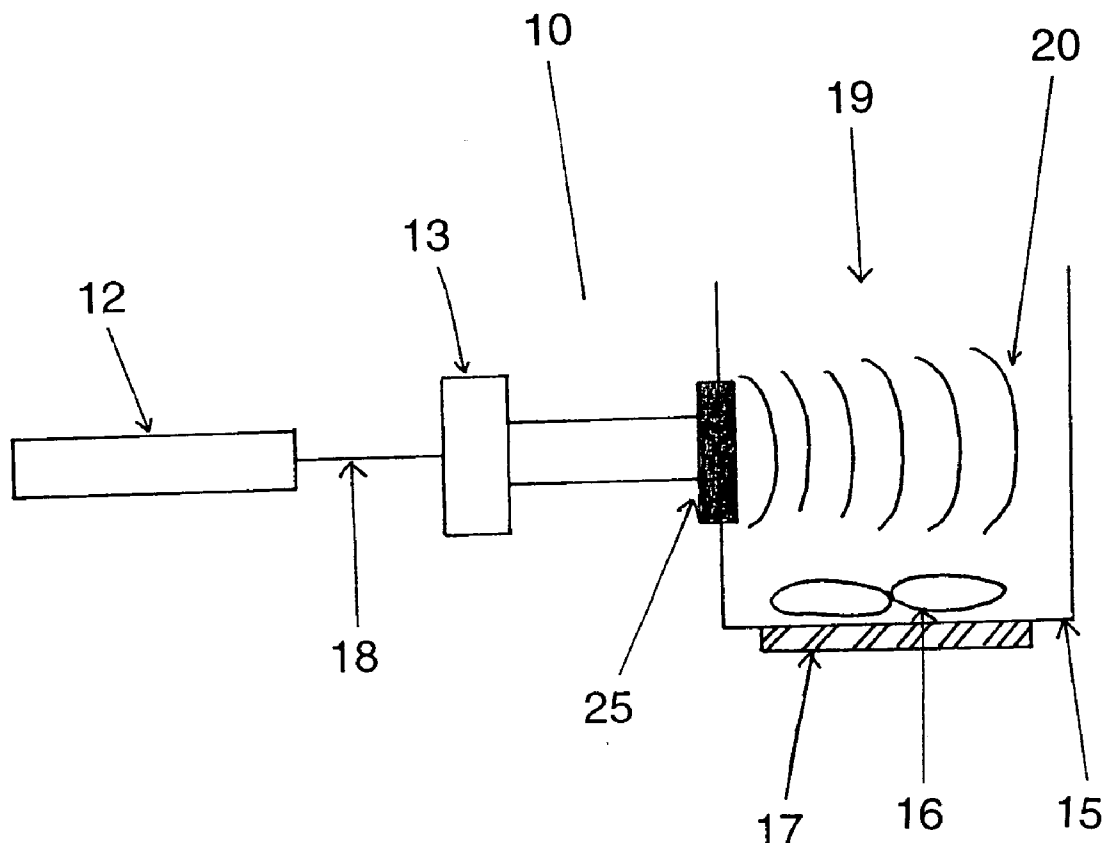
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Stephen T. Flock, Arvada, CO (US)**Publication Classification**(51) **Int. Cl.⁷** **C25D 7/12**(52) **U.S. Cl.** **204/157.15**

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8011 Candle Lane
Houston, TX 77071 (US)(21) Appl. No.: **10/739,680**(22) Filed: **Dec. 18, 2003****Related U.S. Application Data**(63) Continuation-in-part of application No. 09/546,065,
filed on Apr. 10, 2000, now Pat. No. 6,673,214.(57) **ABSTRACT**

The present invention provides methods, devices and systems for increasing the energy of biologically active molecules in vitro or in vivo by utilizing electromagnetic energy, inductively-applied electromagnetic energy or magnetic energy. Such methods, devices and systems also can be used for inducing or accelerating the rate of a reaction by increasing energy of the reactants.



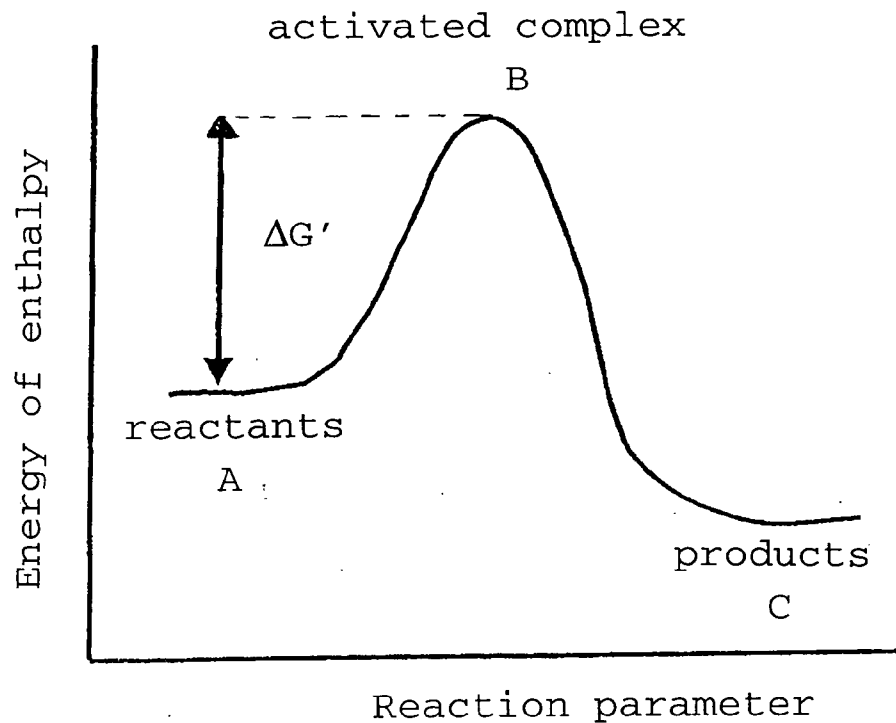


Fig. 1A

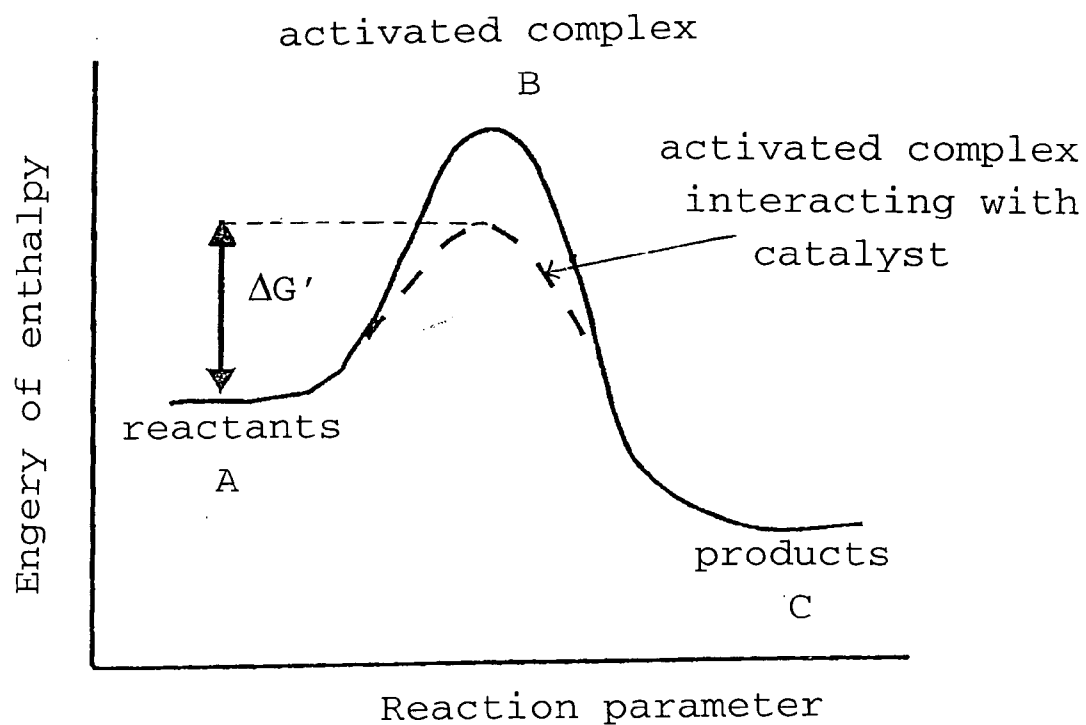


Fig. 1B

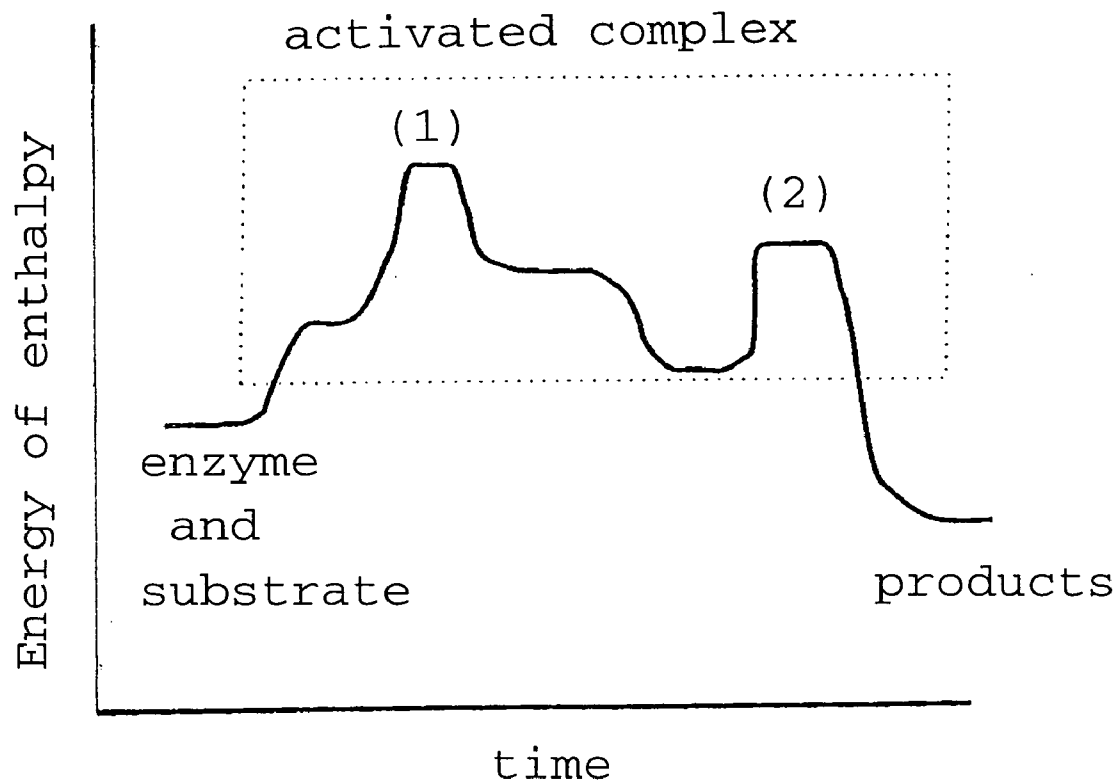


Fig. 1C

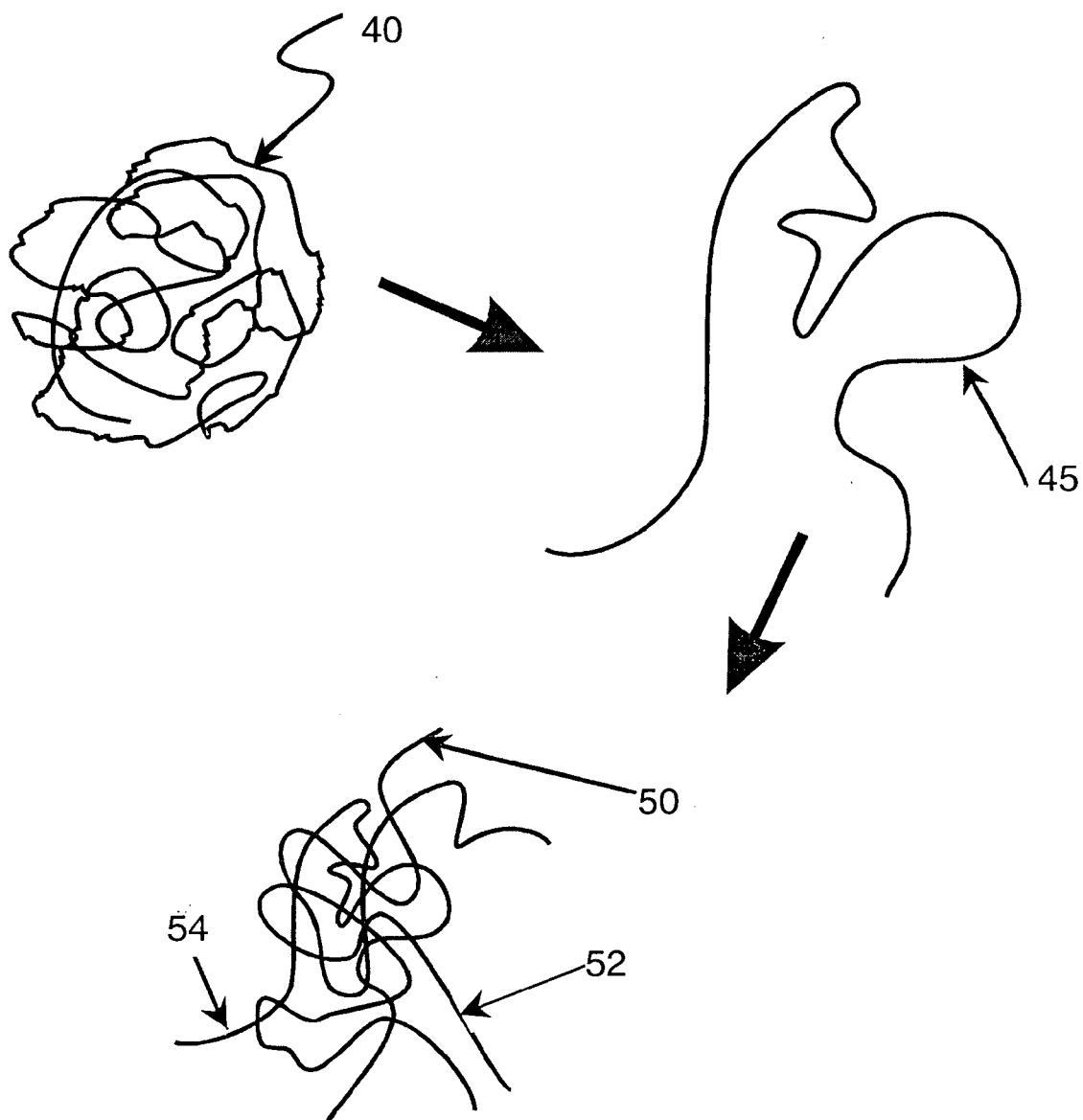


Fig. 2

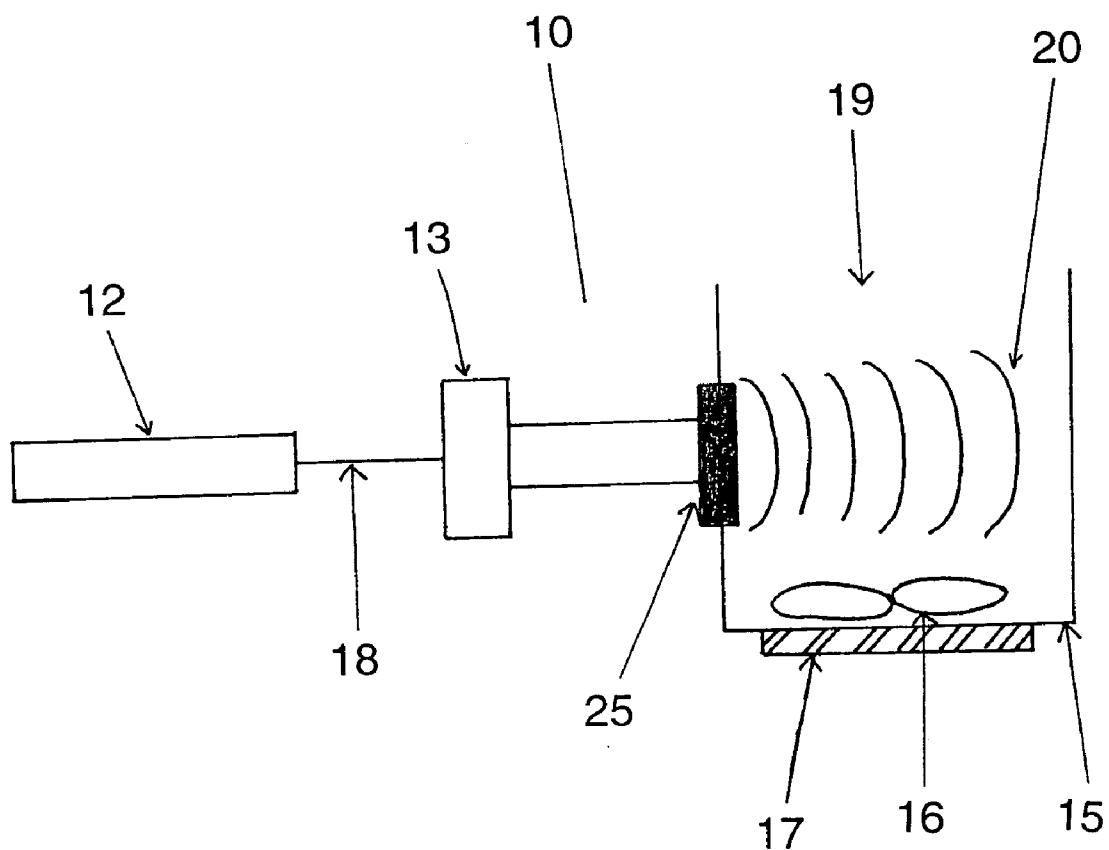


Fig. 3A

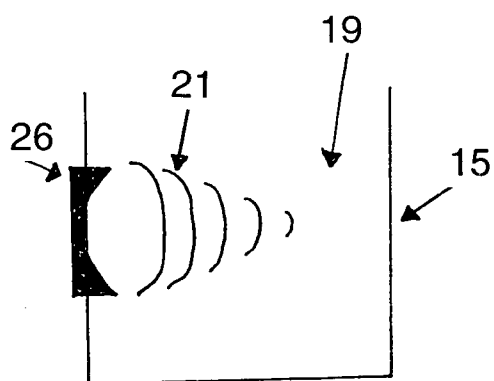


Fig. 3B

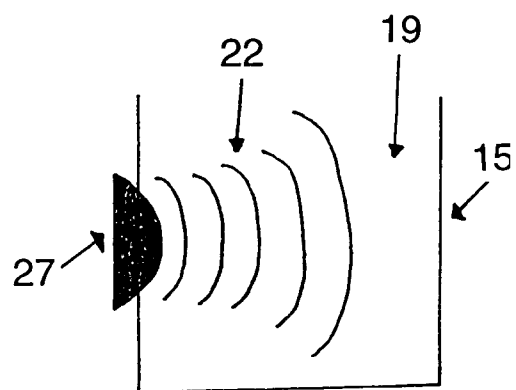


Fig. 3C

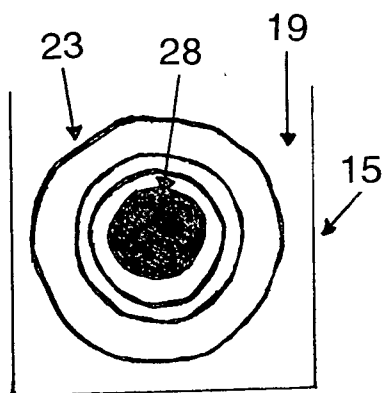


Fig. 3D

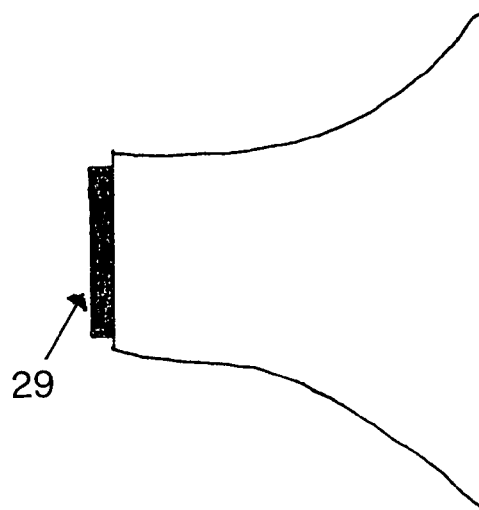


Fig. 3E

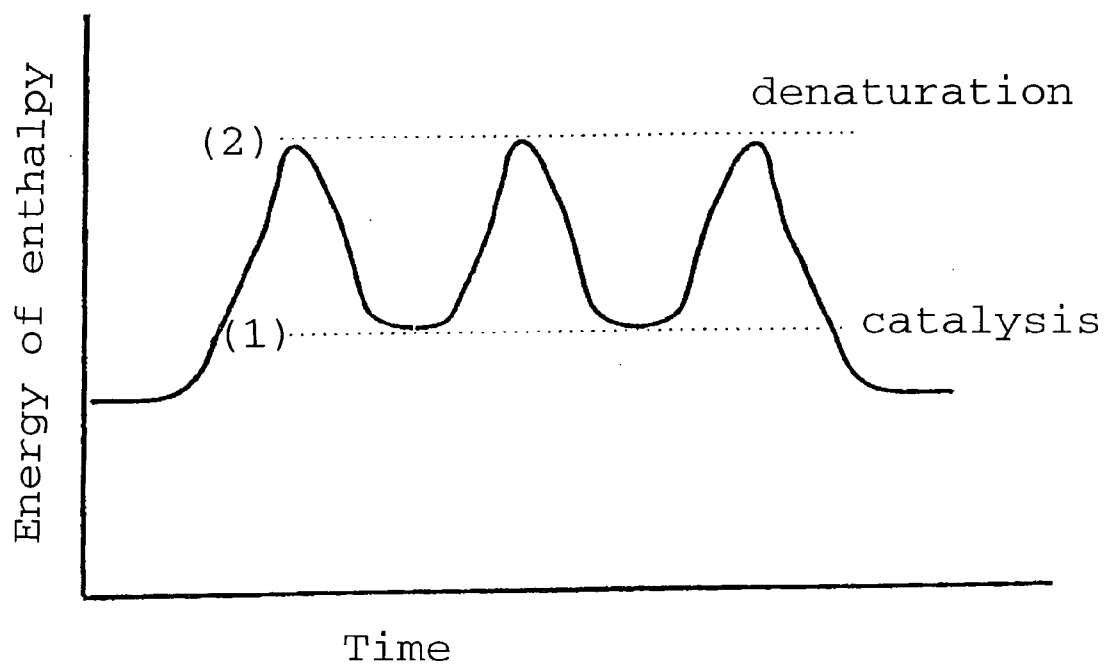


Fig. 4

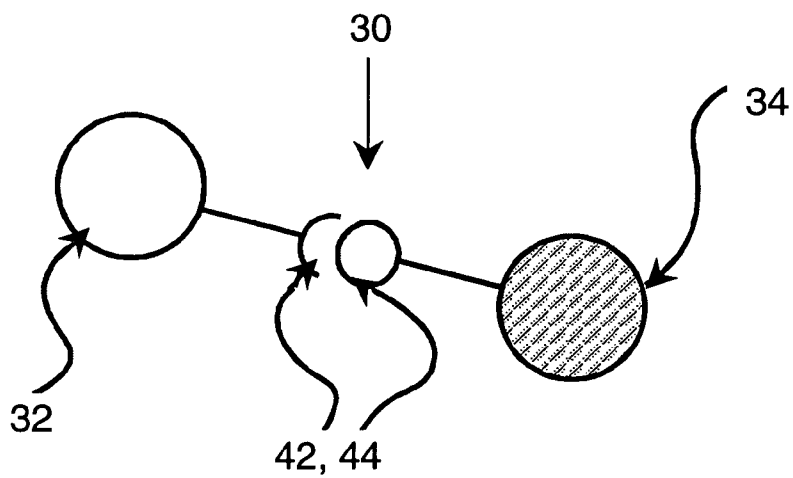


Fig. 5A

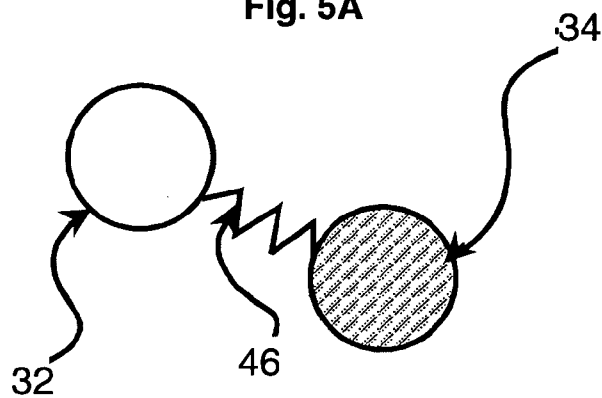


Fig. 5B

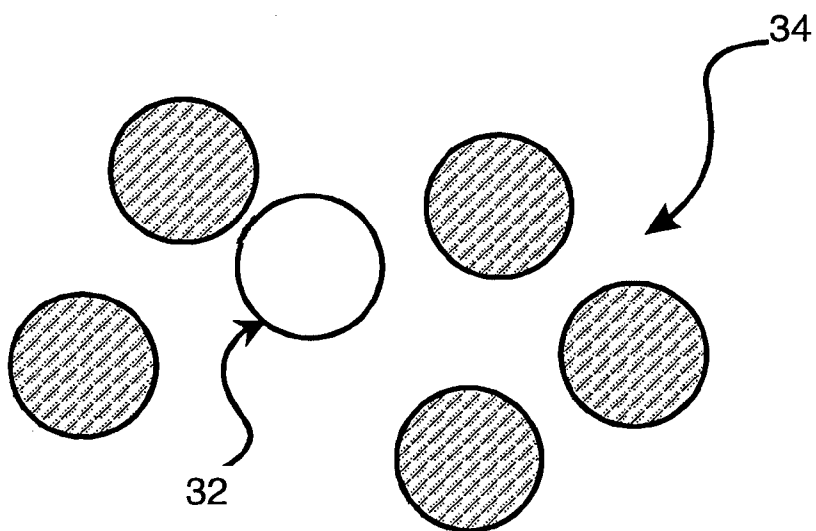


Fig. 5C

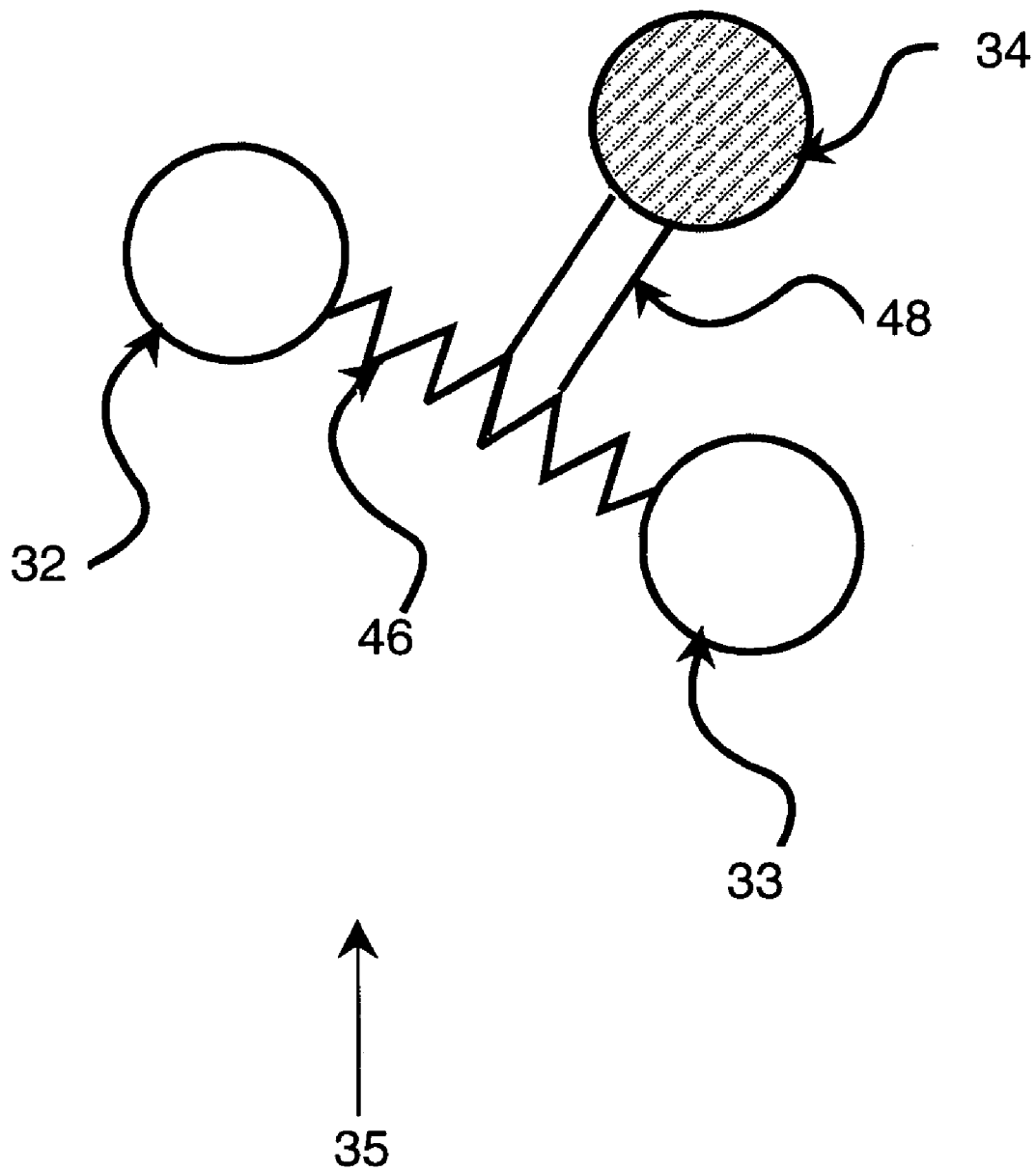


Fig. 5D

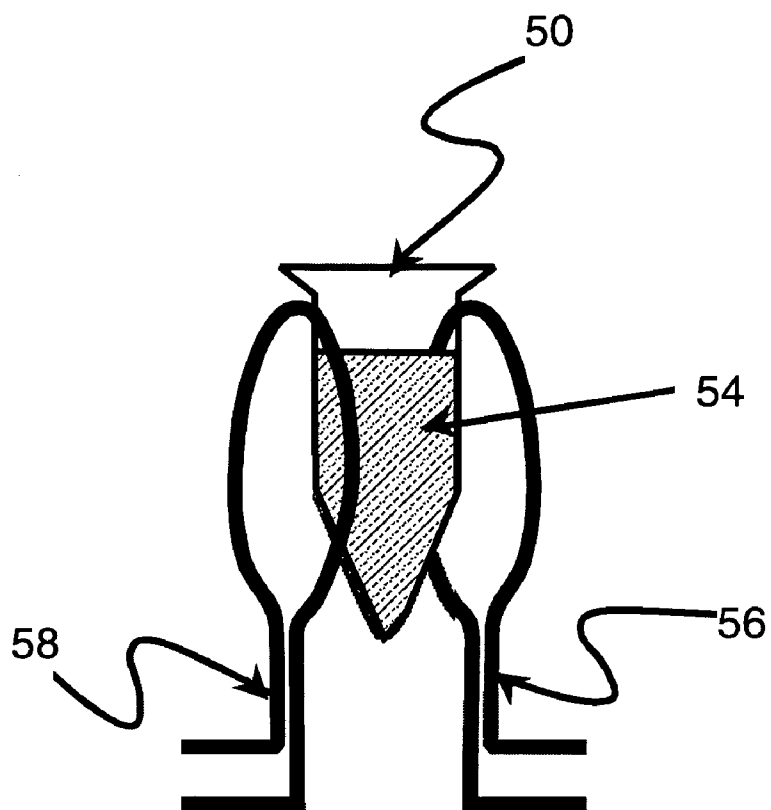


Fig. 6A

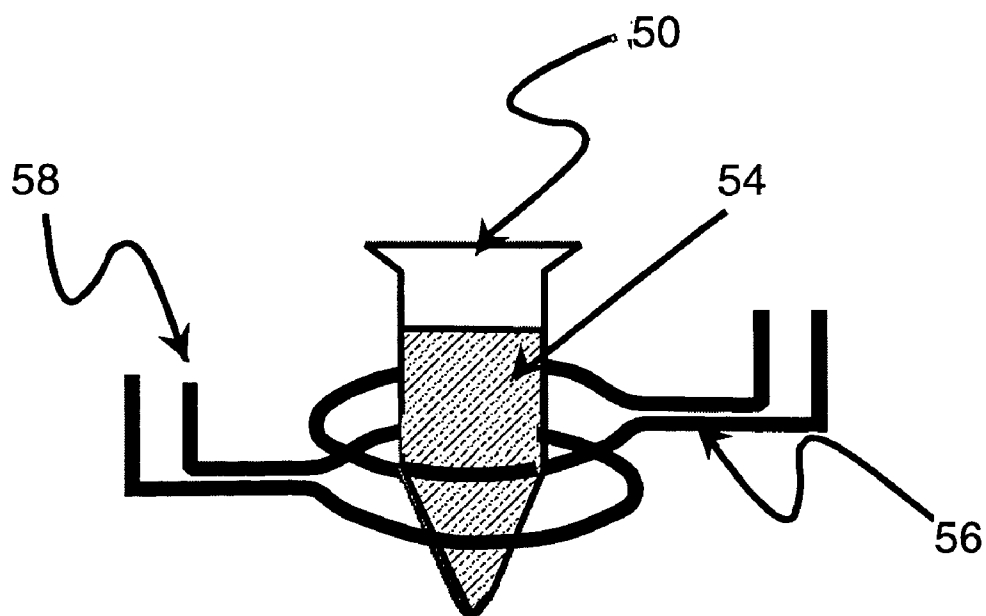


Fig. 6B

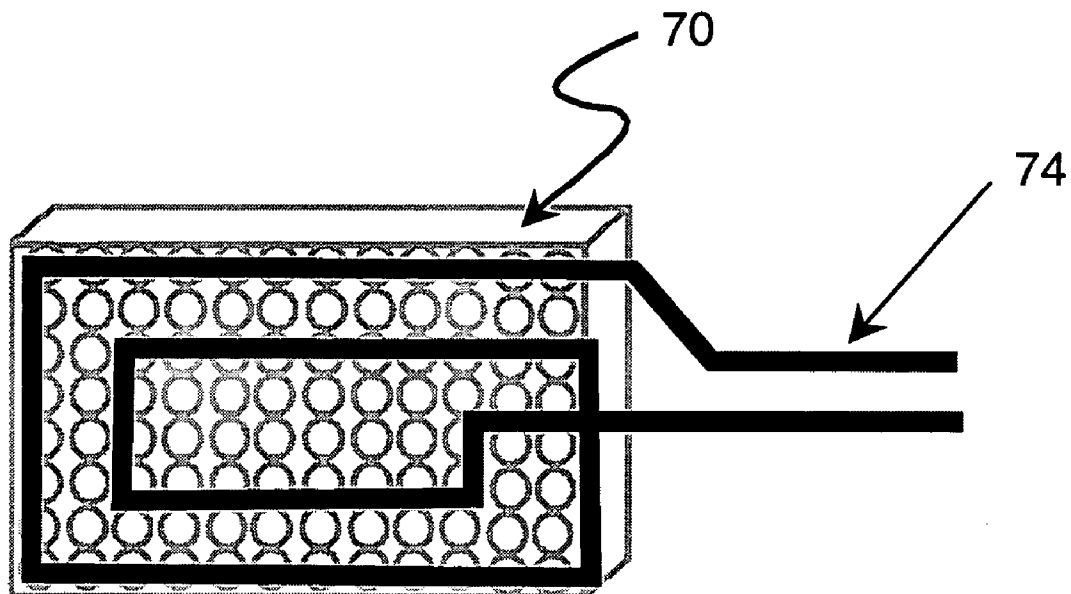


Fig. 6C

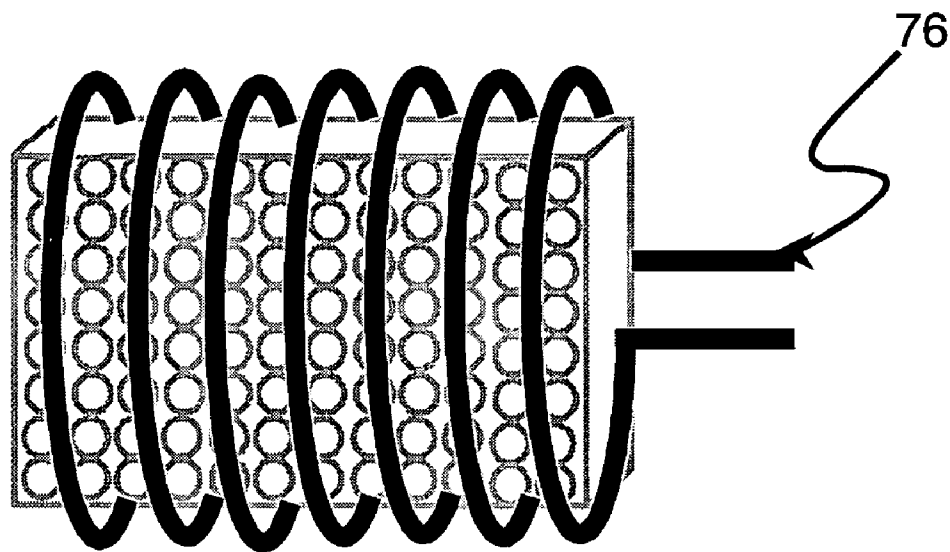


Fig. 6D

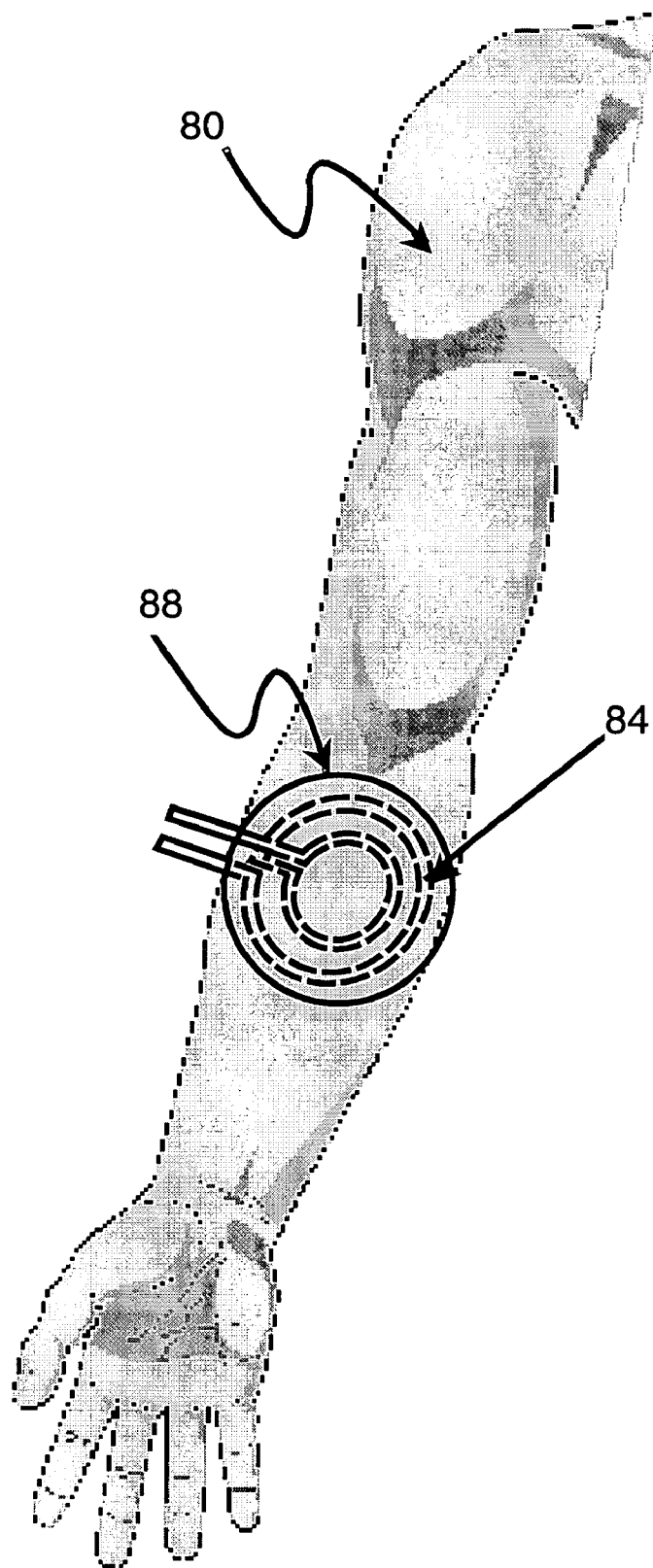


Fig. 7

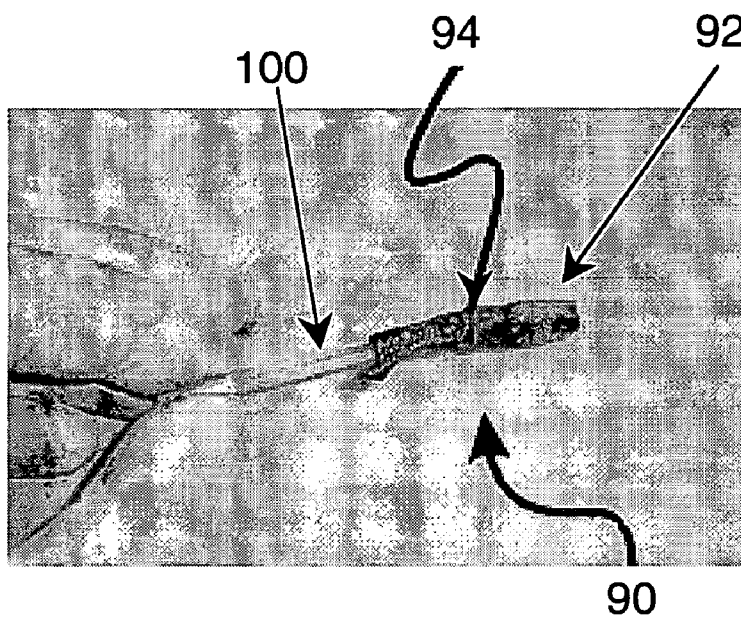


Fig. 8A

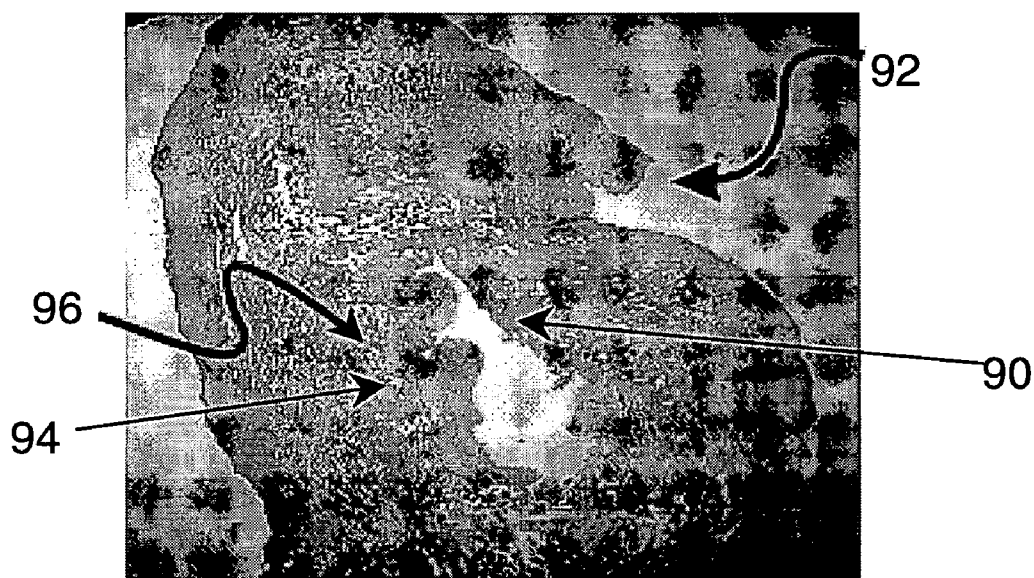


Fig. 8B

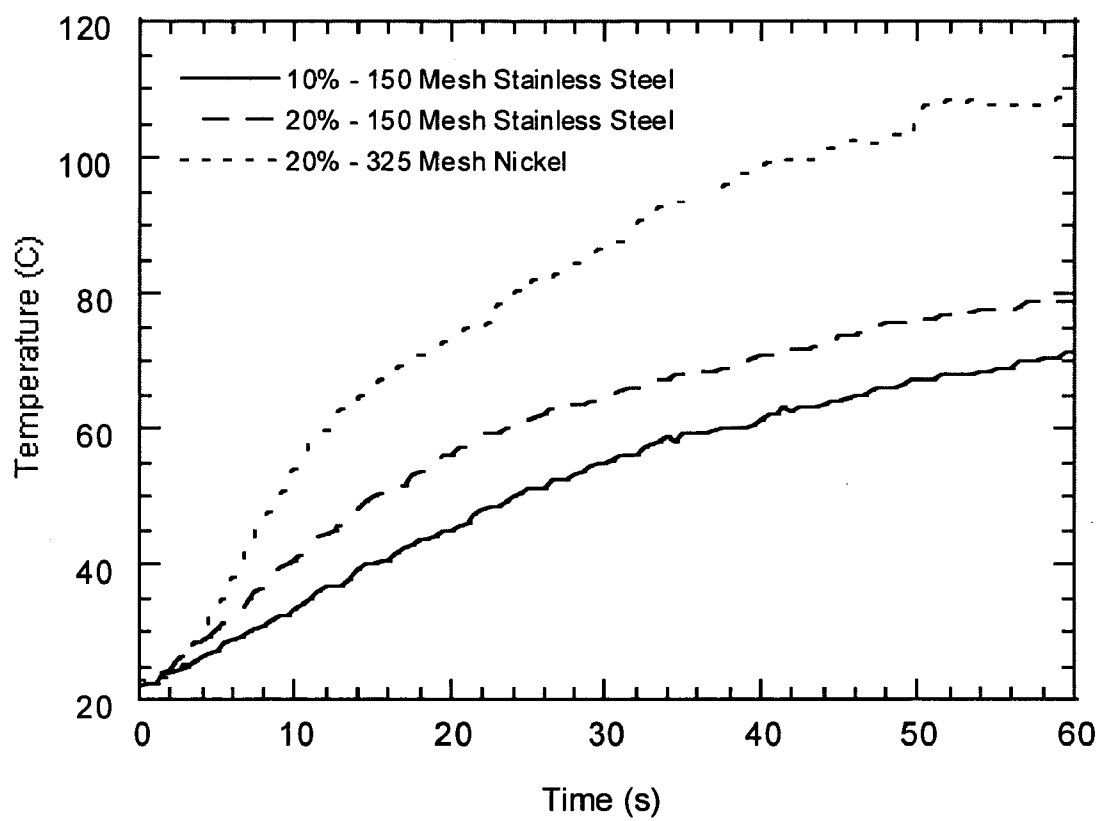


Fig. 9

ENERGY ENHANCED REACTION CATALYSIS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation-in-part of non-provisional patent application U.S. Ser. No. 09/546,065, filed Apr. 9, 2000, which claims benefit of provisional patent application U.S. Serial No. 60/128,444, filed Apr. 8, 1999, now abandoned.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention relates generally to the fields of biochemical reaction and biomedical physics. More specifically, the present invention relates to methods and devices used for adding energy to molecules thereby accelerating a biochemical reaction.

[0004] 2. Description of the Related Art

[0005] In any given chemical reaction, the equilibrium of the reaction is given by the difference in ΔG^0 for the reaction. The equilibrium concentrations of substrate (A) and product (C) are determined by their difference in free-energy content,

$$\Delta G^0 = -RT \ln K_{eq}$$

[0006] where R =gas constant= $8.3 \text{ JK}^{-1} \text{ mol}^{-1}$, T =absolute temperature and K_{eq} =equilibrium constant for the reaction (FIG. 1).

[0007] Heat, when added to a reaction, will alter the free energy content of the reaction and therefore will shift the equilibrium of the reaction. Also, electromagnetic energy, by translating absorbed energy into translational motion, can enhance chemical reactions. However, there is an activation energy barrier between the substrate (A) and the product (C) which is given by ΔG^* . This ΔG^* represents the change in free energy that must be put into the system to reach the transition state, i.e., the energy that must be overcome for the reaction to proceed (FIG. 1A).

[0008] Similarly, the reaction rate is affected by energy of activation, E_a , that reflects the amount of energy that must be added to a reaction for the reactants to reach the transition state. The Arrhenius rate equation describes this reaction rate and is given by:

$$k = A \exp(-E_a/RT)$$

[0009] where A =pre-exponential factor and E_a =activation energy. Given then, that $\ln(k) = \ln(A) - E_a/RT$, a plot of $\ln(k)$ vs $1/T$, gives intercept A and slope $-E_a/R$. If E_a is high, only a portion of the molecular encounters are energetic enough to result in reaction, but, if it is low, a high proportion are energetic enough to react and the rate coefficient is large. Thus, if the activation energy can be lowered in some way, the reaction proceeds more rapidly.

[0010] Anything that stabilizes the transition state relative to reactants will decrease the free energy of activation and, therefore, increase the reaction rate (FIG. 1B). A catalyst lowers the activation energy of the rate determining steps thereby speeding up the reaction. The role of the catalyst is to permit the formation of a transition state of lower energy, i.e., higher stability relative to reactants, than that for

non-catalyzed reactions. The catalyst itself does not participate in the reaction stoichiometry and, thus, is not consumed, and cannot affect the equilibrium position of the reaction. Pauling expressed that stabilization of the transition state of a reaction by an enzyme suggests that the enzyme has a higher affinity for the transition state than it does for the substrate or products. The free energy of activation, ΔG^* , therefore, is reduced during catalysis and is much smaller compared to that without the catalyst.

[0011] In order to undergo a chemical reaction, a reactant or reactants must first gain energy (activation energy) to form an activated complex before they can proceed forward to a state, e.g., products, of different energy or enthalpy. Often, enzymes are used to help the reaction take place, that is, catalyze the reaction. Enzymatic catalysts are believed to lower ΔG^* by replacing a single step of large ΔG^* with several smaller steps of lower ΔG^* (FIG. 1C). The free energy of activation, ΔG^* , is broken into smaller increments by the stepwise action of the enzyme catalyst which first forms an enzyme-substrate complex, ES, then reaches a first intermediate transition state and a second intermediate transition state and finally proceeds to the subsequent product. Thus, enzyme mediated catalysis is extremely efficient for reactions with large ΔG^* because each incremental step is composed of a smaller transition energy of activation.

[0012] Heat can also be used to speed up the reaction rate. Biochemical reaction experiments involving thermal denaturation on human serum albumin show that the protein denatures at $55 \pm 3^\circ \text{C}$. with a total change in free energy for unfolding of $42 \text{ kJ} \cdot \text{mol}^{-1}$ and the protein aggregates, i.e. clusters together with itself and other proteins, after unfolding (1). It is also known that the presence of particular chemical species can stabilize or destabilize proteins against thermal denaturation. It is the biochemical reaction of protein denaturation followed by aggregation that is hypothesized to be the underlying mechanism of laser-tissue welding, as in U.S. Pat. No. 5,713,891. Lasertissue welding is more fully described in a series of patents, e.g., U.S. Pat. Nos. 6,583,117, 6,391,049, 6,323,037, 6,211,335 and 5,929,044.

[0013] Denaturation of DNA can be achieved using thermal energy or radiative energy [6]. Radiative energy can be used to break the hydrogen bonds and also induce specific motions in the DNA molecule. It has been shown [7] that vibrational excitation is efficacious in promoting endoergic reactions. By adding kinetic energy to the molecules, the energy barrier E_a can be overcome.

[0014] DNA has hydrogen bonds between the two strands. For every adenine and thymine base, there are two hydrogen bonds, $\text{O} \dots \text{H}$ and $\text{N} \dots \text{H}$. For every cytosine and guanine base, there are three hydrogen bonds, two $\text{O} \dots \text{H}$ and one $\text{N} \dots \text{H}$. These hydrogen bonds hold the DNA together in the form of a double helix. If these bonds are broken, the DNA unwinds and denatures into two complementary strands. The ionic OH and NH bonds have energies on the order of 111 and 93 kcal/mol respectively, while the $\text{O} \dots \text{H}$ and $\text{N} \dots \text{H}$ hydrogen bonds have energy on the order of about 1 kcal/mol and, therefore, are easily broken.

[0015] Catalytic reactions often rely on specific interactions among various reactants or between the catalyst and reactants. The shape and/or size of molecules can sometimes determine where the molecule has an affinity to localize and

bind. For example, a particular protein can interact with a particular target molecule when the protein is folded into its "native" state; when unfolded, or denatured, it typically cannot interact with the target molecule in spite of the fact that the protein still has the same peptide sequence.

[0016] The particular desirable or deleterious protein-protein, protein-DNA or protein-molecule interaction depends on the correct binding of the molecules. Efficiency of binding or of reacting can be increased by increasing the rate at which the molecules "bump" into each other. This is typically a function of concentration and kinetic energy in the system. Alternatively, binding or reacting can be altered by a change in conformation of one or both of the molecules. A conformational change may increase the exposure of the binding sites on each molecule or may alter the shape of each molecule such that they can approach each other more closely.

[0017] For example, it is generally accepted that blocking the flow of sodium through the sodium channel in nerve cell membranes makes the nerve inexcitable by local action currents. Changes in the shape and caliber of the sodium channel determine what anesthetics are efficacious. The shape and caliber of the channel can be changed by, inter alia, action potentials, toxins and local anesthetics that bind to gating receptor sites. Some local anesthetics bind to a channel protein binding site, thus rendering the sodium channel blocked.

[0018] The polymerase chain reaction (PCR) is an enzyme-catalyzed technique used for in vitro and in situ amplification of specific DNA sequences. The process goes in receptive cycles: denaturing whereby the DNA of interest is denatured for about 4 minutes at 94° C., annealing whereby the appropriate part of the DNA strands are annealed to the primers, i.e., the antisense DNA fragment of interest, at 50° C. for about 2 minutes and extending whereby a heat stable enzyme called Taq-DNA-polymerase (Taq) polymerizes the individual DNA bases, deoxyribonucleotides, for 3 minutes at 72° C. This cycle is repeated for N times, e.g., ~20-30 times, with more primers and nucleotides added.

[0019] For the following cycles, heating parameters may be modified slightly, for example, denaturing for 1 min. at 94° C., annealing for 2 min. at 50° C. and extending for 3.5 min at 72° C. Additionally, the heating parameters for the last cycle also are typically different, for example, denaturing for 1 min. at 94° C.; annealing for 2 min. at 50° C.; and extending for 10 min at 72° C. As a result, the DNA of interest is amplified by 2^N .

[0020] For the heating protocol example above, the total time for denaturation is $4+(20 \times 1)+1=25$ minutes with additional time needed for cooling, if $N=20$. If it were possible to denature and cool the DNA more efficiently, a significant timesaving would result. Additionally, the part of the PCR cycle that involves the highest temperatures would be eliminated and thermal breakdown of the reaction buffer materials would be reduced. Furthermore, increasing the reaction rate of the annealing and extension phases of PCR would also add a timesaving.

[0021] Inductive heating [3] is a non-contact process whereby electrical currents are induced in electrically conductive materials or susceptors by a time-varying magnetic

field. The current ultimately gives rise to ohmic or Joule heating. Generally, induction heating is an industrial process often used to weld, harden or braze metal-containing parts in manufacturing where control over the heating process and minimized contact with the workpiece are critical.

[0022] The theory of induction heating involves radiofrequency power coupled to a conducting element, such as a coil of wire, which serves to set up a magnetic field of a particular magnitude and spatial extent. The induced currents or Eddy currents flow in the conductive materials in a layer referred to as the skin depth (δ), given by:

$$\delta = \sqrt{2\rho/\Omega\mu},$$

[0023] where Ω is frequency (rads/s), ρ is resistivity (ohm-m) and μ is the permeability (Webers/amp/m) which is the product of μ_0 , the permeability of free space and μ_r , the relative permeability of the material.

[0024] The magnetic permeability of a material is quantification of the degree to which it can concentrate magnetic field lines. However, the permeability is not constant in ferromagnetic substances like iron, but depends on the magnetic flux and temperature. The skin depth of 1 MHz electromagnetic radiation in copper at room temperature is 0.066 mm and in 99.9% iron is 0.016 mm.

[0025] The consequence of current flowing is Joule, or I^2R , heating. The skin-depth formula leads to the conclusion that, with increased frequency, the skin depth becomes smaller. Thus, higher frequencies favor efficient and uniform heating of smaller components. In certain situations localized heat can also be generated through hysteresis losses or frictional heating, referred to as dielectric hysteresis heating in non-conductors, as the susceptor moves against physical resistance in the surrounding material.

[0026] Consideration of Joule heating alone results in a formula for the power-density P (W/cm³) in the inductively-heated material:

$$P = 4\pi H^2 f \mu M,$$

[0027] where H is the root-mean-square magnetic field intensity (A/m), f is frequency (Hz), M is a unitless power density transmission factor that depends on the physical shape of the heated material and skin depth and diameter of the part to be heated. M , which is equal to the product of F and d , where F is a transmission factor and d is the diameter of the object inductively coupled to the magnetic field, can be shown to be maximally about 0.2 when the object diameter is 3.5 times the skin depth and when certain other assumptions are made. Thus, for a given frequency there is a diameter for which the power density is a maximum; or equivalently, there is a maximum frequency for heating a part of a certain diameter below which heating efficiency drops dramatically and above which little or no improvement of heating efficiency occurs. It can also be shown that the power density of inductively heated spheres is much higher than solid spheres of the same material.

[0028] It is apparent from the current industrial uses of inductive heating, that using this technique to heat macroscopic solid pieces of electrically conducting metal is relatively easy, although requiring large and expensive power supplies and solenoid-type coils. However, heating small, i.e., <1 mm, particles is difficult; in fact, powdered metals are used in some high-frequency transformers as the powder

does not inductively heat to any extent and so the transformer stays cool during operation. It's an interesting result that it is possible to economically heat microscopic particles in vivo, and use inductive coupling to transform biological materials.

[0029] Only a few examples of the use of in vivo inductive heating are found in the medical literature. The oldest example of use of therapeutic inductive heating is in hyperthermia of cancer whereby large metallic "seeds" are inductively heated using a coil external to the body. Smaller seeds consisting of dextran magnetite particles in magnetic fluid have been used to treat mouse mammary carcinoma by hyperthermia [4]. Hyperthermia always involves temperatures of about 43° C. that are below the threshold for protein denaturation. U.S. patent application Ser. No. 2002/0183829 describes inductively heating solid metal stents positioned in diseased blood vessels for the purpose of killing proliferating smooth muscle cells in restenosing blood vessels.

[0030] There are few examples in the medical literature of the use of in vitro inductive heating. U.S. patent application No. 20020061588 described the use of induction heating to heat nanocrystals coupled to DNA to locally denature DNA for the purpose of hybridization [5]. U.S. patent application No. 20020119572 describe using an external electromagnetic field to alter the property of a protein to which is attached a susceptor, such as a chromophore or metallic or semi-conducting nanoparticle. The susceptor serves to absorb the ambient electromagnetic field.

[0031] U.S. Pat. No. 6,348,679 discloses compositions used in bonding two or more conventional materials where the interposed composition consists of a carrier and a susceptor, which may be at least in part composed of certain proteins. This application applies only to conventional substrates, such as films or wood, and so issues such as biocompatibility and extraneous thermal damage are not relevant. U.S. Pat. No. 6,323,037 describes the addition of an optical "energy converter" to the solder mixture such that incident optical energy will be efficiently and preferentially absorbed by the solder which subsequently effects a tissue weld. Similar optical susceptors are described in U.S. Pat. No. 6,530,944.

[0032] The prior art is deficient in the lack of effective means of accelerating a biochemical reaction, in vitro or in vivo, by adding electromagnetic energy inductively to the reactants therefore to increase the frequency at which reactants reach transition states of reaction. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

[0033] The present invention provides methods, devices and a system for increasing the energy in biomolecules and biologically active molecules. Electromagnetic energy is applied to these molecules resulting in an increase of energy therein. Such an energy increase within the biomolecules and/or the biologically active molecules can be used to drive reactions or to result in a change of state of the molecules. Additionally, a transducer may be used to transfer energy to the reactants, which results in heating of the reactants, thereby leading to an increased rate of reaction. In some cases, this method may enable the reaction.

[0034] The present invention is directed to a method for accelerating a reaction of at least one biologically active

molecule. Electromagnetic energy is applied to inductively generate an ambient electromagnetic field around the biologically active molecules. Energy is transferred from the ambient electromagnetic field to the biologically active molecule(s) to increase energy thereof thereby accelerating the reaction.

[0035] The present invention also is directed to a method of accelerating a biochemical reaction. At least one electromagnetic field transducer is associated with at least one biochemical reactant comprising the biochemical reaction. Radiofrequency energy is applied to inductively generate an electromagnetic field and the reactant(s). Energy from the electromagnetic field is transferred to the reactant(s) via the electromagnetic field transducer to increase the energy of the biochemical reactant(s) thereby accelerating the biochemical reaction.

[0036] The present invention is directed further to a device for inductively heating biomolecules or biologically active molecules. The device comprises a radiofrequency power supply, an electromagnetic field transducer and a means for inductively applying the radiofrequency energy to the biomolecules or biologically active molecules.

[0037] The present invention is directed further still to a system for inducing a biochemical reaction. The system comprises a radiofrequency power supply, a means for inductively applying electromagnetic energy to the reaction, and a reactive composition. The reactive composition comprises at least one biomolecule or biologically active molecule and an electromagnetic field transducer associated therewith.

[0038] Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention briefly summarized above may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

[0040] FIG. 1A depicts reaction coordinates as a function of the free energy.

[0041] FIG. 1B shows reaction coordinates as a function of the free energy in the presence of a catalyst.

[0042] FIG. 1C shows reaction coordinates as a function of the free energy in the presence of an enzyme. The intermediate transition state (1) and (2) are indicated.

[0043] FIG. 2 is a diagram depicting conformational changes in a protein upon denaturation and aggregation.

[0044] FIG. 3A shows device used for enhancing a PCR reaction. FIGS. 3B-3E show that different shaped radiant energy absorbing targets within the walls of a reaction

chamber in the device in 3A, or inside the reaction solution, produce different pressure waves.

[0045] FIG. 4 is an energy diagram demonstrating alternating cycles of radiant energy for multi-step reactions such as the optically enhanced PCR. Energy reaching level (2) is sufficient to separate the strands of DNA. The energy is then reduced to a lower level (1) that favors a second reaction in the sequence by catalysis. Level (1) is not great enough to denature the molecular products. This cycle is repeated to produce long strands of DNA.

[0046] FIGS. 5A-5D depict different ways to position an inductive transducer in proximity to the reactant.

[0047] FIGS. 6A-6D depict different arrangements of an induction applicator positioned in proximity to a reaction chamber (FIGS. 6A-6B) and to a multiwell screening plate (FIGS. 6C-6D) in order to enhance in vitro biochemical reaction rates.

[0048] FIG. 7 shows a coil type applicator, substantially made out of an electrically non-conducting material, positioned on the arm of a subject for in vivo use. The coil inductor antenna 84 is housed within the applicator. This device could be used in vivo to induce conformational changes in reactants coupled with transducer species.

[0049] FIG. 8A shows the anastomosis or fusion of two sheep arteries with a fusion composition using inductively-applied radiofrequency energy to fuse the arteries.

[0050] FIG. 8B depicts a histologic section across the lumens at the fusion juncture of the sheep carotid arteries.

[0051] FIG. 9 compares temperature over time for heating fusion compounds using a commercially available induction power supply

DETAILED DESCRIPTION OF THE INVENTION

[0052] In one embodiment of the present invention, there is provided method accelerating a reaction of at least one biologically active molecule comprising applying electromagnetic energy to inductively generate an ambient electromagnetic field around the biologically active molecule(s); and transferring energy from the ambient electromagnetic field to the biologically active molecule(s) to increase energy thereof thereby accelerating the reaction of the biologically active molecule(s).

[0053] Further to this embodiment the method may comprise associating an electromagnetic field transducer with the biomolecules or biologically active molecules prior to the application of electromagnetic energy. The electromagnetic field transducer may comprise matter which has a non-zero electrical conductivity. Such matter may be diamagnetic, paramagnetic, or ferromagnetic. The electromagnetic field transducer may function as an antenna.

[0054] The matter may be an ionomer, a conducting polymer, an alkali metal, a transition metal, a lanthanide, or a metalloid or a combination thereof. In such instances the matter may comprise matter is colloidal or non-colloidal gold, silicon, cadmium selenide, cadmium sulfide, ruthenium, indium phosphide, indium arsenide, gallium arsenide, gold maleimide, gallium phosphide, hydroxysuccinimidyl gold, nickel-copper, nickel-palladium, palladium-cobalt,

nickel-silicon, stainless steel, iron oxide, ferrite, titanium, Phynox, palladium/cobalt alloys, nitinol, titanium, titanium alloys, zirconium, gadolinium, aluminum oxide, dysprosium, cobalt alloys, nickel, gold, palladium, or tungsten or alloys thereof. Furthermore, the matter may be matter is a metal nano- or micro-particle, a semiconducting nano- or micro-particle, a magnetic nano- or micro-particle, a polystyrene encapsulated metal particle, a buckminsterfullerene, or a liposome-encapsulated metal particle.

[0055] In aspects of this embodiment the electromagnetic energy is radiofrequency energy. The radiofrequency energy may have a frequency from about 100 kHz to about 40 GHz. The electromagnetic field may be inductively generated via an antenna or series of antennae. The antenna may comprise at least one coil of electrical conductor. Examples of an electrical conductor are a solid wire or hollow tubing. Representative examples of an antenna are a single coil antenna, a double coil antenna or a solenoid antenna. Alternatively, the electromagnetic energy is applied via a magnet.

[0056] Additionally, in all aspects of this embodiment the biologically active molecule may be a protein, a lipid, nucleic acids, or a carbohydrate or combination thereof. The biologically active molecule may be in a tissue or in a tissue system. Alternatively, the biologically active molecule may be in vitro.

[0057] In an aspect of this embodiment, the increase in energy may accelerate a biochemical reaction in which the biologically active molecules are reactants. The biochemical reaction further may comprise a pharmaceutical, a biologic, other biologically active molecules, diagnostics, or biological markers or a combination thereof. The biochemical reaction may be enzyme catalyzed.

[0058] In a related aspect the biologically active molecule has a conformational change during the biochemical reaction. An example of conformational change is denaturation, protein unfolding or protein refolding or a combination thereof. Such biochemical reactions may be a polymerase chain reaction or an enzyme-linked immunosorbent assay.

[0059] In a related embodiment there is provided a method of accelerating a biochemical reaction comprising associating at least one electromagnetic field transducer with at least one biochemical reactant comprising the biochemical reaction; applying radiofrequency energy to inductively generate an electromagnetic field; and transferring energy from said electromagnetic field to said reactant(s) via said electromagnetic field transducer to increase the energy of said biochemical reactant(s) thereby accelerating the biochemical reaction.

[0060] In this embodiment the biochemical reactant(s) may be biologically active molecules comprising proteins, lipids, nucleic acids, or carbohydrates or a combination thereof. Additionally, the biochemical reaction further may comprise a pharmaceutical, a biologic, other biologically active molecules, diagnostics, or biological markers or a combination thereof. The reactants may be located in a tissue or a tissue system. In this embodiment the radiofrequency energy, the biochemical reactants and their location, the electromagnetic field transducers, and the antennae are as described supra.

[0061] In another embodiment of the present invention, there is provided a device for inductively heating biologi-

cally active molecules comprising a radiofrequency power supply; an electromagnetic field transducer; and a means for inductively applying the radiofrequency energy to the biologically active molecules.

[0062] In this embodiment the power supply generates radiofrequency energy from about 100 kHz to about 40 GHz. Additionally, the means for inductively applying radiofrequency energy may be an antenna. The antenna may be the electromagnetic field transducer as described supra. Also, the electromagnetic field transducer, the biologically active molecules and the locations thereof are as described supra.

[0063] In yet another embodiment of the present invention, there is provided a system for inducing a biochemical reaction comprising a radiofrequency power supply; a means for inductively applying electromagnetic energy to the reaction, and a reactive composition comprising at least one biologically active molecule; and an electromagnetic field transducer associated therewith.

[0064] In this embodiment the biologically active molecules may be proteins, lipids, nucleic acids, or carbohydrates or a combination thereof. The biologically active molecules may be located in vitro or in a tissue or tissue system. Furthermore, the system may comprise a pharmaceutical, a biologic, other biologically active molecules, diagnostics, or biological markers or a combination thereof. The radiofrequency power supply, electromagnetic field transducers, the means for inductively applying the radiofrequency energy, and the antenna(e) are as described supra.

[0065] Provided herein are methods and devices to accelerate a biochemical reaction by adding energy conductively or inductively to one or more of the reactants or to all of the reactants in a mixture. The reactants may comprise at least one biologically active molecule. The biochemical reaction may be a catalyzed reaction, such as, but not limited to, a reaction catalyzed by an enzyme. The biochemical reaction can be either a liquid-phase reaction or a solid-phase reaction.

[0066] Biologically active molecules may be biomolecules or otherwise biologically active molecules which are naturally occurring in a living organism or those which can have an influence on molecules in a living organism. Typically, such molecules may be found in or around cells and tissues or may be supplied to living organisms, cells and tissues to achieve a desired effect or response. Examples of biomolecules include proteins, carbohydrates or lipids found in cells or tissues. The biomolecules may be, although not limited to, structural such as tissue structures comprising elastin or collagen or structural cellular components such as actin, myosin, or ribonucleoprotein particles. The biomolecules may be involved in catalysis, e.g. as enzymes, or may be reactants such as protease susceptible proteins, metabolized lipids. Other examples of biologically active molecules include biological response modifiers, antigens, protease inhibitors, other enzymes, and metabolic inhibitors.

[0067] The reactants may be placed within a device comprising a reaction vessel, e.g., a microvessel or a reaction chamber. Alternatively, the device may comprise an applicator or patch which can be placed against biological tissue or affixed onto an individual. The device further comprises means of delivering the electromagnetic energy or mechanical energy described herein to the reactants.

[0068] In one device radiant energy may be supplied in such a way that reactants within the vessel are exposed to a free energy increase in the system which causes increased vibration or rotation in molecular groupings or causes electrons to shift to excited states making the molecules more reactive. A catalyst present in or attached to the reaction vessel in such a way that the reactants are in contact with the catalyst will then be in position to stabilize transition states of the reactants, thereby improving the chances that the reaction will proceed forward.

[0069] The reaction vessel may contain at least one reactant and one enzyme that reacts in a step-wise manner to generate products. Radiant energy of a certain wavelength or other energy is delivered to the reaction vessel in such a way that the reactants are activated so the first step catalytic reaction proceeds at a faster pace. A second wavelength and/or energy is then applied to the reaction such that a second step, which requires a different energy of activation, can then take place. These steps may be repeated for cyclic reactions. These reaction vessels can be used for enhancing a PCR reaction.

[0070] The energy added to the reaction may be electromagnetic energy or mechanical energy. Examples of electromagnetic energy are radiant energy, radiofrequency energy, microwave energy or magnetic energy. The electromagnetic energy may be generated by a source which provides radiant energy with wavelength from about 200 nm to about 20,000 nm. The electromagnetic energy may have a frequency in the region of the electromagnetic spectrum of about 100 kHz to about 40 GHz. Alternatively, radiofrequency energy may be added inductively to the reactants. Mechanical energy may be a pressure wave.

[0071] Since the energy imparted to a system by photonic energy increases the free energy of the system, then the frequency at which molecules reach a transition state is increased. In the presence of a catalyst, this transition state is stabilized relative to reactants and the forward rate of reaction is favored. However, energy must be added to the reaction system with care so that the reactants are not simply denatured or destroyed to produce useless species.

[0072] Controlling the level of energy imparted into a system is important. Radiant energy from lasers, and other forms of energy described herein, such as microwaves and radiofrequency waves, may be controlled easily to deliver an appropriate measure of energy. By imparting small increments of energy to a reaction mixture, in the form of radiant energy for example, the stepwise transitions may be taken advantage of and the undesirable reactions and denaturation avoided.

[0073] It is contemplated that when radiant energy is provided to an enzyme catalyzed reaction mixture such that energy, in the form of heat, is delivered locally in the immediate environment of the molecule, a thermal acceleration of the rate of reaction results. This local heating effect is only transient, on the order of less than microseconds, in the case of a pulsed laser. However, as the transition state for the reaction is maintained only on the order of 10^{-13} seconds, it is of sufficient length to result in an increase in the frequency at which these molecules reach their transition state. In the presence of a catalyst, this transition state is stabilized and the reaction proceeds. Short and high-energy pulses of radiant energy can be used resulting in little heating of the surrounding medium.

[0074] Alternatively, a continuous wave or pulsed laser is used to heat the surrounding medium rapidly in such a way that the heat involved in the reaction increases rapidly. A small reaction volume can then be cooled rapidly so that unstable products or side reactions are minimized. This approach is particularly useful when cyclic reactions are necessary where rapid heating and cooling steps may take place in relatively small volumes.

[0075] Also, energy may be added to the system using microwaves or radiofrequency waves in the same manner. Radiofrequency waves are of particular interest because they are capable of causing the vibrational effects in the medium containing the reactant without heating of the applicator. In effect, radiofrequency waves may be incorporated into a heating element that does not heat up the element itself during use. In this case, the heating element could always be kept cold while it transmits energy to the reaction medium in direct contact with or near to it.

[0076] Still alternatively, short and repeated pulses of radiant energy are directed at the molecular species thereby causing an increased level of excitation. This approach takes advantage of the fact that molecular species absorb photonic energy, which increases their intrinsic rate of rotation or vibration and the likelihood that inner shell electrons will be promoted to outer shells, thereby creating a less stable structure. These events result in an increased frequency of the molecules to attain a transition state. In the presence of catalyst, the transition state is stabilized and therefore the reaction proceeds accordingly.

[0077] The present invention also contemplates that pulsed or continuous wave radiant energy can be used to directly excite molecules to which light-absorbing complexes are attached. By attaching indocyanine-green dye to molecular species which can come into contact with, or come into close proximity to, the DNA, primers or nucleotides of interest, energy then is added to the reactants by transference from these dye-conjugates molecules or simply by the dye itself upon the absorption of a pulse of radiant energy from a laser. For example, an alexandrite laser tuned to emit energy with a wavelength of 795 nm could be used. The benefit of such an approach over gross heating of the reaction mixture is that the average power introduced into the reaction mixture is less where pulsed irradiation is used so that the reaction mixture does not heat up significantly.

[0078] The methods provided herein can increase the rate at which a group of molecules reaches a different molecular configuration from initial configuration, such as a molecular configuration present during a transition state in a biochemical reaction. The introduction of heat to molecules, such as a proteins, causes denaturation and aggregation. As demonstrated in FIG. 2, heat applied to the protein 40 causes it to unfold or denature to a different configuration 45. The denaturated protein 45, in the presence of other denaturated molecules 52 and 54, of the same or different type, tend to aggregate, thus effecting a bond therebetween.

[0079] These methods include activating molecules or molecular groups through energy absorption from infrared radiation. Absorption of infrared radiation by biomolecules can be broken down into three regions. In the near-infrared (NIR, 800 nm-1.5 microns), an OH group stretching vibration is near 7140 cm^{-1} (1.4 microns) and an NH stretching vibration is near 6667 cm^{-1} (1.5 microns). In the mid-

infrared, 4000 to 1300 cm^{-1} (2.5-7.7 microns) is the "group frequency region", while 1300 to 650 cm^{-1} (7.7-15.4 microns) is the "fingerprint region". Absorptions in the near infrared region depend on the functional group present and not the complete molecular structure or in the mid-infrared region depend on single-bond stretching and bending vibrations.

[0080] The methods described herein can be used to denature DNA. Radiant energy with a wavenumber of about $1550\text{--}1800\text{ cm}^{-1}$ induces in-plane double-bond vibrations of bases, with a wavenumber of about $1275\text{--}1550\text{ cm}^{-1}$ induces base deformation motions coupled through glycosidic linkages to sugar vibrations, with a wavenumber of about $1050\text{--}1275\text{ cm}^{-1}$ induces phosphate and sugar vibrations, and with a wavenumber of about $750\text{--}1050\text{ cm}^{-1}$ induces vibrations of the sugar-phosphate backbone and bases. All of these infrared spectral domains are in the infrared region of the electromagnetic spectrum with wavelengths of about 5.5-12.5 microns. Therefore motions in specific bonds can be induced with infrared electromagnetic energy. For example, the 1225 cm^{-1} and 1084 cm^{-1} bands, i.e., asymmetric and symmetric stretching vibrations of nucleic acids, can be targeted by lasers emitting long-wavelength radiant energy; e.g., a CO_2 laser.

[0081] Additionally, as the energy of the hydrogen bonds between the two strands of DNA, at about 1 kcal/mol, is about $100\times$ lower than that for ionic OH and NH bonds, the bonds are easily broken. Continued exposure to temperatures of 75°C . causes the hydrogen bonds between strands of DNA to break. Similarly, hydrogen bonds can be broken by radiant energy photons with a wavelength of about 32 microns or less. This is in contrast to the ionic OH and NH bonds which require photons with wavelengths of about 307 nm or less. Consequently, one can break hydrogen bonds in DNA without causing breaks in the DNA strands themselves, i.e. between the base-sugar or base-phosphate bonds, by using radiant energy with a wavelength greater than about 300 nm. However, by using infrared radiant energy, any damage to bonds other than hydrogen bonds, such as weak covalent bonds with energies about 3-7 kcal/mol, can be avoided.

[0082] Furthermore, the methods described herein can induce specific motions in a DNA molecule. For example, the OH stretching vibration near 1.4 microns and the NH stretching vibration near 1.5 microns could be excited with radiant energy produced by, for example, an Nd:YLF or Nd:glass laser. Thus, irradiating the DNA with 1.4-1.5 micron radiant energy prior to the hydrogenbond-breaking step done with radiant energy at a longer wavelength would increase the reaction rate. Similarly, by irradiating the denatured DNA with 1.415 micron radiant energy after the denaturation step would increase the reaction rates of the annealing and extension step. In this way, different wavelengths may be used to differentially or combinatorially enhance the conversion of a species from one to another.

[0083] The present invention also provides mechanical energy to enhance reaction rates. Compressional or tensile propagating pressure waves can be produced by irradiating biological media with pressure waves produced by acoustic transducers, such as piezoelectric crystals or ultrasonic transducers, or radiant energy produced by a pulsed laser or by irradiating absorbing materials placed in intimate acous-

tic contact with the tissue of interest. Shock waves, i.e., propagating pressure waves moving in a medium at velocities greater than the speed of sound in the medium, can be created with significant amplitudes, quantified in bars or J/cm³, while propagating pressure waves travelling at the velocity of sound typically are of less amplitude.

[0084] Using shock waves in the present invention can be beneficial as they may be more efficient in inducing bond breaks or molecular vibrations and/or rotations. The physical characteristics of propagating pressure waves not only result from the energy source used to create the waves, but also are a consequence of the medium in which they propagate. Thus a propagating pressure wave can transform into a shock wave within a short distance while propagating in water, for example.

[0085] A pressure wave with an amplitude of 300 bars can provide about 0.72 kcal/mol of energy. This is sufficient to break hydrogen bonds, but not enough to break covalent or ionic bonds. The ability to break bonds and induce vibrations results from not only the amplitude of the wave, but also the temporal characteristics. Further, the addition of an appropriate catalyst to any of these enhanced reactions would favor stabilization of transition molecules and therefore further increase the forward reaction rate.

[0086] Alternatively, it can be beneficial to de-gas the reaction mixture, or introduce hyperbaric inert gases, e.g. N₂, in the reaction mixture to maintain a sealed reaction vessel in order to modulate the extent to which cavitation bubbles are produced. These bubbles propagate and carry significant amounts of energy to damage biological media, such as DNA. On the other hand, if they are controllably produced, they may be used to beneficially add energy to the reaction mixture.

[0087] When propagating pressure waves are produced by pulsed laser radiant energy impinging on an absorbing target, different shaped targets may be beneficial. For example, different shaped targets within the walls of the reaction chamber, or inside the reaction solution, can produce pressure waves that can be beneficial. Multiple lasers can also be used to produce multiple propagating pressure waves impinging on the reaction mixture from different directions. It is beneficial to continually mix the reaction mixture and place the reaction chamber on a controllable heating/cooling element to maintain the temperature of the reaction solution in a region suitable for the process to take place.

[0088] Such device comprises a radiant energy source for heating the reaction, a microvessel containing the reactants and a cooling chamber. Optionally, a catalyst can be included. The vessel alternates between heating and cooling cycles allowing standard PCR reaction to proceed. Alternatively, an optional reaction chamber, without the energy absorbing target/transducer, may be used to directly delivery energy to the reactants for either local heating or for increasing the energy state of the reactants thereby increasing the frequency at which the reactants reach transition state.

[0089] As shown in FIG. 3A, the device 10 comprises a pulsed laser source 12, expanding/focusing optics 13, radiant energy absorbing target/transducer 25, a reaction chamber 15 containing stir bars 16, and a heater/cooler 17 in contact with the reaction chamber 15. The pulsed laser

source 12 generates radiant energy in the form of a laser beam 18 which is expanded and focused by the optics 13. The laser beam 18 is absorbed by the target/transducer 25 and transduced into propagating pressure waves 20 in the reaction chamber 5 to interact with a reaction mixture 19. Optionally, the reaction mixture 19 may be stirred so reactants interacting with the propagating pressure waves 20 do so substantially uniformly. Additionally, the heater/cooler 17 may heat/cool the reaction mixture 19 to further promote the reaction.

[0090] FIGS. 3B-3E demonstrate that different shaped radiant energy absorbing targets within the walls of the reaction chamber 15, or inside the reaction solution, produce different pressure waves. FIG. 3B shows a target/transducer 26 within the wall of the reaction chamber 15, as in FIG. 3A, having a concave face presented to the reaction mixture 19. The target/transducer 26 transduces the laser beam (not shown) into pressure waves 21 that decrease as they propagate. Alternatively, FIG. 3C shows a target/transducer 27 within the wall of the reaction chamber 15, as in FIG. 3A, with a convex face presented to the reaction mixture 19. The target/transducer 27 transduces the laser beam 18 into pressure waves 22 that increase as they propagate.

[0091] FIG. 3D demonstrates that the target/transducer 28 may be in the reaction chamber 15 with the reaction mixture 19. The pressure waves 23 propagate uniformly throughout the reaction mixture 19.

[0092] FIG. 3E depicts a target/transducer 29 which may be placed in the wall, may be part of the wall, or may be in the reaction chamber.

[0093] The methods of the present invention may be used to increase or to decrease the probability of two molecules interacting in vivo by use of electromagnetic energy or propagating pressure waves. For example, radiant energy with particular parameters can be used to produce intense propagating pressure waves in tissues, which can serve to transiently alter the shapes of molecules, in turn altering the way molecules interact in those tissues. Alternatively, direct absorption in the molecule of electromagnetic energy, e.g., infrared radiation, can cause vibrations and rotations, thereby enhancing the ability of the molecule to approach and/or bind to a particular site. Taken to an extreme, molecules that do not normally interact by virtue of their mutually repulsive conformations can be made more reactive by the addition of energy that alters their conformations.

[0094] Transport of molecules across membranes or other tissue barriers may be enhanced using the methods described herein. For example, irradiation of a membrane or of the skin results in transient changes in the molecular configuration of its constituents. Molecules that subsequently contact the irradiated molecules will have an accelerated rate of binding or reacting as compared to those in non-irradiated tissue. This is applicable to drug permeation through membranes or tissue barriers.

[0095] For example, one of the rate limiting steps in permeation of compounds through membranes or tissue barriers is the rate of initial binding to local receptors. For locally acting anesthetics, such as lidocaine, permeation is in part related to the rate of local binding to receptors. Thus, local anesthetics will bind irradiated membrane receptors at an accelerated rate over non-irradiated receptors. Therefore,

these anesthetics will permeate the membrane at an accelerated rate, as long as they are able to bind to their receptors at an accelerated rate. This concept thus improves the rate at which drugs can be delivered across tissue and membrane barriers.

[0096] The methods described herein can increase the speed and efficiency of a polymerase chain reaction (PCR) or of an enzyme linked immunoassay (ELISA). The yield of PCR products may also increase and the amount of reactants used reduced by minimizing or eliminating heating of the PCR reaction solution. Reducing or eliminating the heat involved in denaturation also reduces or eliminates the need for thermostable enzymes.

[0097] For example, in the reaction catalyzed by the enzyme DNA polymerase, a sample of chromosomal or other DNA is present in the reaction chamber with an oligonucleotide primer and the catalyst. In the first step, laser radiation is added to the chamber to increase the energy of the reaction such that the strands of the DNA are denatured and separated either locally or for the greater length of the molecule. When the laser energy is removed, either completely or in between pulses, the strands of DNA will then re-anneal. In the presence of the oligonucleotide primer which matches part of the sequence of a strand of DNA, the primer will compete for binding sites to the strand of denatured DNA. If the primer is in excess relative to the concentration of the sister DNA strand, more primer will bind as opposed to strands re-annealing.

[0098] In the presence of DNA polymerase, additional nucleotides will be added to the end of the primer such that a new DNA strand is produced. Imparting radiant energy to the reaction chamber will increase the level of activation of the reactants in this case, and the reaction will proceed at a faster rate. Thus, there are two opportunities to use radiant energy in this reaction.

[0099] A certain wavelength and sufficient energy is used to first denature the DNA. When the energy is removed or reduced, competitive binding of the oligonucleotide may take place. In the catalytic step, the same or different wavelength radiation will be used, but this time a lower energy that will not denature the double stranded DNA, will then accelerate the rate of the DNA polymerase reaction. By cycling the two wavelengths and/or energies, the reaction may be repeated multiple times (**FIG. 4**).

[0100] The present invention provides methods of accelerating a biochemical reaction by inductively transferring energy to the reactants. Specifically, the present invention provides a method of using radiofrequency electromagnetic energy to enhance biochemical reactions so that vibrations, rotations, and/or particular configurations or orientations of the reactants are induced whereupon a desirable reaction takes place. These methods are especially suitable for use in biochemical reactions both in vitro and in vivo.

[0101] Transducers accelerate a biochemical reaction via efficient coupling to an ambient electromagnetic field resulting in a transfer of or conversion of energy. Transducer species used in this way comprise matter with a non-zero electrical conductivity such as, but not limited to, electrical conductors, semiconductors or ionomers. They may be ferromagnetic, diamagnetic or paramagnetic. Transducers can be linked to or positioned in close proximity to reactant

molecules, such as, although not limited to, proteins. Thus, transducer species function to transfer energy between an ambient radiofrequency electromagnetic field and the reactants to enhance the ability of the molecules to absorb ambient radiofrequency electromagnetic energy and so proceed with the desirable biochemical reaction. Transducer species also may function as antennae to inductively transfer energy. In some cases, the energy may be converted to another energy form, such as heat or kinetic energy.

[0102] One or more of the reactants taking part in the biochemical reaction may have a molecular or macroscopic absorbing species linked to it or in close proximity to it, for the purpose of enhancing the transfer of energy from the electromagnetic field to the reactants. The molecular transducer may be an ionomer, conducting polymers, alkali metals, transition metals, lanthanides, or metalloids. Examples of such ionomers include, without limitation, styrenated ethylene-acrylic acid copolymer or its salts, sulfonated polyesters and their salts, sulfonated polystyrene and its salts and copolymers, polyacrylic acid and its salts and copolymers, hydroxy/carboxylated vinylacetateethylene terpolymers, functionalized acrylics, polyesters, urethanes, epoxies, alkyds, latex, gelatin, soy protein, casein, collagen, and other proteins, alginate, carrageenan, starch derivatives, ionic polysaccharides, and the like, and sodium polystyrene-sulfonate.

[0103] The transducer may be a metal nanocrystal or metal nano- or micro-particles, semiconducting nano- or micro-particles, magnetic nano- or micro-particles, polystyrene-encapsulated metal particles, buckminsterfullerenes, or liposome-encapsulated metal particles. Metals or metal compounds may be colloidal or non-colloidal gold, silicon, cadmium selenide, cadmium sulfide, ruthenium, indium phosphide, indium arsenide, gallium arsenide, gold maleimide, gallium phosphide, hydroxysuccinimide gold, nickel-copper, nickel-palladium, palladium-cobalt, nickel-silicon, stainless steel, iron oxide, ferrite, titanium, Phynox, palladium/cobalt alloys, nitinol, titanium, titanium alloys, zirconium, gadolinium, aluminum oxide, dysprosium, cobalt alloys, nickel, gold, palladium, tungsten, or alloys of materials from this group.

[0104] The inductive transducers may be positioned in proximity to the reactant using different configurations. In **FIG. 5A** a reactant **32** and transducer **34** are linked using, for example, avidin and biotin **42,44** bound in no particular order. The resulting molecular species **30** can be prepared from a kit or may be used as components in a kit. During use the molecular species **30** are mixed with appropriate reagents and the avidin-biotin complex **42,44** binds the reactant **32** and transducer **34** in close proximity to one-another so that the transducer **35** can link the ambient radiofrequency energy (not shown) to the reactant **32** to accelerate the reaction. As shown in **FIG. 5B**, the reactant **32** and transducer **34** are linked with a chemical bond **46**.

[0105] Alternatively, **FIG. 5C** shows the reactant **32** and transducer **34** are not chemically linked, but are in proximity with one-another through stochastic processes such as diffusion. In another configuration, **FIG. 5D** demonstrates how the inductive transducer **34** is positioned proximate to reactants **32,33** to controllably break the chemical bond **46** between them. The transducer **34** is bonded to the dimer **35** formed by chemically-linked reactants **32,33** with the same

bond **46** (not shown) or a different bond **48**. Upon activation of the transducer **34**, the bond **46** in the dimer **35** is broken.

[0106] The present invention also provides devices for inductively transferring energy to reactants, in vivo or in vitro, thereby accelerating a biochemical reaction. The reactants may comprise biomolecules such as proteins, lipids, nucleic acids, or carbohydrates or a combination thereof. The reaction mixture may further comprise a pharmaceutical, a biologic, other biologically active molecules, diagnostics and other biological markers. The reaction mixture may or may not comprise inductive transducer matter.

[0107] An induction applicator can be positioned proximate to a reaction vessel, such as a reaction chamber or a multiwell plate, to enhance an in vitro biochemical reaction rate. The reaction vessel may substantially be made of an electrically conductive material and/or magnetic material, whereupon it would heat during the application of an electromagnetic field. Alternatively, the reaction vessel may be of an electrically non-conducting material which is transparent to the applied electromagnetic field.

[0108] The induction applicator may comprise one or two antennae. The antenna may be one or more coils of electrical conductor. The electrical conductor is a solid wire or hollow tubing. The induction coils may be a single loop coil, a double loop coil or a multi-loop coil, such as a solenoid, and may be positioned adjacent to and proximate to the reaction vessel. Alternatively, the reaction vessel may be positioned within the induction coil(s). An optional second coil can increase field strength and/or improve field uniformity. The magnitude of the induction field is typically stronger within the turns of the induction coils in contrast to the induction field from the face of an induction coil or antenna.

[0109] In FIG. 6A, an antennae **56** comprising a single-turn circular coil is positioned in close proximity to a reaction chamber **50**, in which a reaction mixture **54** is placed. The antenna **58** shown positioned in proximity to the reaction chamber is optional. FIG. 6B depicts a similar device where the reaction chamber **50** containing reaction mixture **54** is positioned within the turns of the induction coils **56,58**. In FIG. 6C, a multiwell plate **70** is positioned against a flat two-loop coil antenna **74**. Alternatively, in FIG. 6D, the multiwell plate **70** is positioned within the turns of a solenoid type coil antenna **76**.

[0110] The inductive devices of the present invention can be used to accelerate a biochemical reaction rate in vivo. These devices could be used, inter alia, to induce conformational changes in reactants coupled with transducer species in vivo. FIG. 7 depicts a coil type applicator **88**, substantially made out of an electrically non-conducting material, positioned on the arm **80** of a subject. The coil inductor antenna **84** is housed within the applicator.

[0111] Accelerated biochemical reactions have multifold beneficial uses in vivo, for example, the fusion of tissue separated through surgery or trauma, e.g., an anastomosis. A fusion composition may be utilized in such an instance. The fusion composition may comprise, although not limited to, a protein found in body tissues and metal particles or ionic species as transducers. The metal particles or ionic species inductively transduce an electromagnetic field to heat applied to the protein in the fusion composition thereby altering its molecular conformation, such as unfolding the

protein, and accelerating a reaction between the denatured or unfolded protein and tissue. Upon completion of the fusion process the protein may refold differently in association with the tissue thereby fusing it.

[0112] It is contemplated that such a fusion composition may be used as an adhesive to reinforce staples or sutures or used in sutureless anastomosis. Optionally, the fusion composition further may comprise a layer of pullulan as a backing or a laminate to function as a tape. Examples of such application may be after a colorectal surgical procedure to reinforce integrity of the suture line or to stop bleeding and protect the puncture wound after dialysis.

[0113] Additionally, in the field of cancer therapy it is known that the efficacy of chemotherapeutic drugs in inducing lethal damage to malignant cells increases with the increasing time that the drug is present adjacent to or within the cells. An ongoing problem in cancer therapy is getting malignant cells to retain chemotherapeutic drugs. It is contemplated that a potentially powerful form of cancer therapy would involve in situ inductive biomolecular alteration or activation of a chemotherapeutic drug/magnetic particle conjugate [8] which would serve to make the cancerous tissue retain the drug.

[0114] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

[0115] Heat Mediated Reaction Catalysis

[0116] A reaction involving test molecules is enhanced by transiently and repetitively raising the temperature of the surrounding environment i.e. the ambient molecules.

[0117] Polymerase Chain Reaction (PCR) reaction

[0118] A PCR mixture including buffers, primers, Taq polymerase and deoxyribonucleotides was prepared as per manufacturer's instructions and placed in a reaction vial. An equal amount of a sample of DNA to be amplified was added to the mixture. The reaction mixture was exposed to the radiant energy produced by a Ho:YAG laser. The laser radiant energy output was configured to produce a spot size at the reaction vial of 5 mm with a 400 microsecond pulse, 10 Hz pulse repetition rate and 200 mJ/pulse pulse energy. The radiant energy, which was absorbed by the reaction mixtures, was arranged to impinge on the lateral side of the reaction vial in a position so that the reaction mixture was irradiated. The irradiation continued for 20 minutes after which the mixture was assayed for DNA amplification. The degree of amplification was compared to non-irradiated control samples demonstrating that greater amplification occurred in the irradiated mixture as compared to controls.

[0119] Enzyme Linked Immunoassay (ELISA)

[0120] An ELISA reaction mixture was prepared in 96-well plates previously seeded with equal numbers of rat cancer cells. The reaction mixture was MTT, which produces a calorimetric change in the presence of viable cells. After adding the assay reaction mixture to the wells in the plate, single wells were irradiated with the radiant energy produced by a Ho:YAG laser. The laser radiant energy output, which was absorbed by the reaction mixtures, was configured to produce a spot size at the surface of the

reaction mixture of 5 mm with a 400 microsecond pulse, 10 Hz pulse repetition rate and 200 mJ/pulse pulse energy. The irradiation continued for 10 minutes, after which a solvent was added and the mixture was spectroscopically assayed for color. The result indicates that a greater degree of color change, i.e., a greater optical density (OD), occurred in the irradiated mixture as compared to controls. Since the wells were seeded with equal numbers of cells, the greater OD is indicative of an enhanced reaction catalysis and not of different numbers of viable cells.

[0121] Alternatively, an ELISA reaction mixture was prepared in 96-well plates previously seeded with equal numbers of rat cancer cells. The reaction mixture was MTT. After adding the assay reaction mixture to the wells in the plate, the entire 96-well plate was exposed to microwave energy by placing the plate within a 500 Watt microwave oven. The microwave irradiation conditions were as follows: the oven was set to the defrost mode, the lowest power possible, and the oven was sequenced on and off repetitively, that is, approximately 2 seconds on and 10 seconds off, for a period of 10 minutes. After the irradiation a solvent was added and the mixture was spectroscopically assayed for color. The result shows that a greater degree of color change or greater OD occurred in the irradiated mixture as compared to non-irradiated controls. Since the wells were seeded with equal numbers of cells, the greater OD is indicative of an enhanced reaction catalysis and not of different numbers of viable cells.

EXAMPLE 2

[0122] Heat Mediated Reaction Catalysis with Added Absorber

[0123] A reaction involving test molecules is enhanced by transiently and repetitively raising the temperature of an absorbing species added to the surrounding environment or reaction mixture.

[0124] A PCR mixture was prepared as per manufacturer's instructions and placed in a reaction vial. An equal amount of a sample of DNA to be amplified was added to the mixture. The enzyme present in the reaction mixture was Taq polymerase. The reaction mixture was exposed to the radiant energy produced by a ruby laser. The laser radiant energy output was configured to produce a spot size at the reaction vial of 5 mm with a 400 microsecond pulse, 10 Hz pulse repetition rate and 200 mJ/pulse pulse energy.

[0125] Indocyanine green (ICG) was solubilized in ultra-pure water and added to the reaction mixture to a final concentration of 1%. The radiant energy, which was absorbed primarily by the ICG, was arranged to impinge on the lateral side of the reaction vial in a position so that the reaction mixture was irradiated. The irradiation continued for 20 minutes after which the mixture was assayed for DNA amplification. The degree of amplification was compared to non-irradiated control samples. The result indicates that greater amplification occurred in the irradiated mixture as compared to controls.

EXAMPLE 3

[0126] Reaction Catalysis by Self-Absorption

[0127] A reaction involving test molecules is enhanced by transiently and repetitively adding energy to the test molecules themselves.

[0128] A PCR mixture including buffers, primers, Taq polymerase and deoxyribonucleotides was prepared as per manufacturer's instructions and placed in 0.1 mm pathlength optical cuvettes. An equal amount of a sample of DNA to be amplified was added to the mixture. The reaction mixture was exposed to the radiant energy produced by an Nd:YLF laser. The laser radiant energy output was configured to produce a spot size at the lateral side of the cuvette of 5 mm with a 400 microsecond pulse, 10 Hz pulse repetition rate and 20 mJ/pulse pulse energy. The radiant energy, which was absorbed by the OH and NH stretching vibrations in DNA at or near 1.4-1.5 microns, was arranged to impinge on of the reaction vial in a position so that the reaction mixture was irradiated. The irradiation continued for 20 minutes after which the mixture was assayed for DNA amplification. The degree of amplification was compared to non-irradiated control samples. The result indicates that greater amplification occurred in the irradiated mixture as compared to controls.

EXAMPLE 4

[0129] Reaction Catalysis by Exposure to Propagating Pressure Waves

[0130] In the present study, a reaction involving test molecules is enhanced by transiently and repetitively exposing the test molecules and reaction mixture to propagating pressure waves.

[0131] A PCR mixture including buffers, primers, Taq, or other DNA polymerase, and deoxyribonucleotides was prepared as per manufacturer's instructions and placed in a reaction cuvette. An equal amount of a sample of DNA to be amplified was added to the mixture. A Q-switched or mode-locked laser, e.g., ruby or Nd:YAG, with a 20 ns pulse duration, 1 mm spot size, 10 Hz pulse repetition rate, and 20 mJ pulse energy was directed on a light absorbing material, such as thermoelectrically cooled black anodized aluminum, in intimate acoustic contact with the reaction cuvette. Impulse transients up to about 1000 bars can be created easily in the reaction cuvette.

[0132] By irradiating the reaction cuvette for several seconds during the denaturation step of the PCR cycle, it was not necessary to heat the reaction mixture. Using a lesser intensity of irradiation during the annealing and extension steps also served to increase the reaction rate. Heating and cooling cycles, as in regular thermally controlled PCR, can optionally be done during each stage using a standard PCR reaction apparatus, such as manufactured by Perkin Elmer Inc.

EXAMPLE 5

[0133] Reaction Catalysis by Exposure to Cavitation

[0134] A reaction involving test molecules is enhanced by transiently and repetitively exposing the test molecules and reaction mixture to cavitation.

[0135] An ultrasonic tissue disrupter, with 5 mm diameter probe tip, was immersed in the MTT-ELISA reaction mixture. In order to minimize heating and yet produce cavitation, the ultrasound was applied with a duty cycle of approximately 2 seconds on and 10 seconds off and the reaction tube was kept in a thermoelectrically cooled plate. After the irradiation, a solvent was added and the mixture

was spectroscopically assayed for color. The result indicates that a greater degree of color change or OD occurred in the irradiated mixture as compared to non-irradiated controls, which is indicative of an enhanced reaction catalysis and not of different numbers of viable cells.

EXAMPLE 6

[0136] Protein Denaturation

[0137] A radiofrequency electromagnetic device, operating at 650 kHz, was constructed. Near this frequency, the skin depth in tissue, using conductivity values for canine skeletal muscle at 1 MHz, is about 205 μm , while for nickel, it is 14 μm . Two solenoid type coils were constructed using 20G solid copper wire. The coils were encapsulated in a Pyrex sleeve through which low-viscosity mineral oil is circulated as a coolant. Two coils had 200 turns of solid copper wire, formed into a solenoid with a diameter of 2.86 cm and width of 0.95 cm. The magnetic intensity within the bore of the coil is calculated to be greater than 100 kA/m, while at approximately 0.5 cm from a single coil face the intensity is calculated to be maximally 0.15 kA/m. Two coils were electronically connected to the radiofrequency power supply and physically arranged with the bore axes parallel and opposing each other with a gap of about 2 cm between the faces of the coils.

[0138] The reactant was ovalbumin at a concentration of 50% (w/v) albumin in 0.9% saline as a high viscosity liquid or 75% (w/v) albumin as a paste. The transducer species was nickel flake, with an average particle size of about 46 micron, mixed into the albumin solution at 5-10% (w/v). The mixture of albumin, saline and nickel, i.e., the fusion composition, had a highly viscous rheological nature. The fusion composition preparation showed visual evidence, e.g., coagulation and change in opacity, and was warm to the touch after 20-30 seconds when placed between the two solenoid coils with the radiofrequency power supply producing about 210 W.

EXAMPLE 7

[0139] Vascular Fusion

[0140] Ex vivo sheep arteries were dissected transversely across the lumen to form sections or were cut longitudinally to form sheets of tissue. The fusion composition described supra was sandwiched between small sections, e.g. 1 cm^2 , of the tissue sheets and placed between the coils as before. Tissue fusion was apparent by observation. The tissues fused together seamlessly and it became difficult to tease apart the two sections with forceps. No effort was made to control temperature; however, overheating was apparent from a color change in the tissue with longer exposure times, i.e., >45 seconds.

[0141] As depicted in FIG. 8A, a fusion composition comprising 5% Ni and 50% albumin (not shown) was placed on the adventitia of one end of a transverse-cut sheep artery 90 and the end of another sheep artery 92 dissected across the lumen was placed over the adventitia of artery 90 and the 200 micron layer of the adhesive fusion composition. A glass rod 100 was used as a support to hold the arteries 90,92 in place. The sample was then positioned between the faces of the opposing coils (not shown) and the sample was exposed for about 30 seconds. The magnetic intensity between the

two coils is theoretically estimated to be about 0.3 kA/m. Fusion, or anastomosis, was visually apparent after about ninety seconds and the fused tissue 94 could not be teased apart with forceps without dissection. Tests were repeated five times with equivalent results.

[0142] The vessels were placed in 10% formalin, sectioned transversely across the fused area and submitted for histological preparation and staining with hematoxylin-eosin. FIG. 8B shows presence of metallic transducer particles 96 at the interface between the two overlapping sections of arteries 90,92 and delineates the margin of tissue fusion 94.

EXAMPLE 8

[0143] A commercially available induction power-supply (Lepel Corp., Edgewood, N.Y.) modified through the addition of internal capacitors to accept a solenoid coil was tested on different fusion compositions. The device produced an average power of about 100 W at a frequency of 400 kHz and a field intensity of 0.3 A/m. The output of the device was coupled into a helical wound coil with an outside diameter of 11 cm made of 11 turns of $\frac{1}{8}$ inch copper tubing.

[0144] The fusion compositions tested contained 50% albumin with a transducer consisting of 10% 150 mesh stainless steel, or 20% 150 mesh stainless steel, or 20% 325 mesh nickel. Each fusion composition was separately positioned within the bore of the coil flush with the surface, and the temperature of the upper surface of the fusion composition was measured with an infrared thermometer (FIG. 9). As expected, nickel heats more efficiently than stainless steel due to its greater magnetic permeability, reaching a threshold temperature of $\sim 70^\circ\text{C}$. within 30 seconds, while stainless steel transducers require double the time.

[0145] The following references were cited herein.

- [0146] 1. Flora et al. Unfolding of Acrylodan-Labeled Human Serum Albumin Probed by Steady-State and Time-Resolved Fluorescence Methods, *Biophys J.*, Vol. 75, pp. 1084-1096 (1998).
- [0147] 2. R. Zare. Laser Control of Chemical Reactions, *Science*, Vol. 279, pp. 1875-1879 (1998).
- [0148] 3. Davies EJ. Conduction and Induction Heating. *Inst. Elect. Eng.* and P. Peregrinus: London (1990).
- [0149] 4. Jordan A. et al. Effects of magnetic fluid hyperthermia (MFH) on C3H mammary carcinoma in vivo, *Int. J. Hyperthermia*. Vol. 13, pp. 587-605 (1997).
- [0150] 5. Hamad-Schifferli et al., *Nature*, Vol. 415, pp. 152-155 (2002).
- [0151] 6. J. Liquier and E. Taillandier. *Infrared Spectroscopy of Nucleic Acids*. In: *Infrared Spectroscopy of Biomolecules*. H. Mantsch and D. Chapman, eds., pp. 131-158, Wiley-Liss: NY (1996).
- [0152] 7. Polanyi JC. *Acc. Chem. Res.* Vol. 5, pg. 161 (1972).
- [0153] 8. Rudge SR, et al. Preparation, Characterization, and Performance of Magnetic Iron-Carbon Composite Microparticles for Chemotherapy. *Biomaterials*, Vol. 21, pp. 1411-1420 (2000).

[0154] Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually incorporated by reference.

[0155] One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present examples along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

What is claimed is:

1. A method for accelerating a reaction of at least one biologically active molecule, comprising:

applying electromagnetic energy to inductively generate an ambient electromagnetic field around said biologically active molecule(s); and

transferring energy from said ambient electromagnetic field to said biologically active molecule(s) to increase energy thereof thereby accelerating the reaction of said biologically active molecule(s).

2. The method of claim 1, further comprising:

associating an electromagnetic field transducer with said biologically active molecules prior to the application of electromagnetic energy.

3. The method of claim 2, wherein said electromagnetic field transducer comprises matter with non-zero electrical conductivity.

4. The method of claim 3, wherein said matter is diamagnetic, paramagnetic, or ferromagnetic.

5. The method of claim 2, wherein said matter is an ionomer, a conducting polymer, an alkali metal, a transition metal, a lanthanide, or a metalloid or a combination thereof.

6. The method of claim 5, wherein said matter is colloidal or non-colloidal gold, silicon, cadmium selenide, cadmium sulfide, ruthenium, indium phosphide, indium arsenide, gallium arsenide, gold maleimide, gallium phosphide, hydroxysuccinimidyl gold, nickel-copper, nickel-palladium, palladium-cobalt, nickel-silicon, stainless steel, iron oxide, ferrite, titanium, Phynox, palladium/cobalt alloys, nitinol, titanium, titanium alloys, zirconium, gadolinium, aluminum oxide, dysprosium, cobalt alloys, nickel, gold, palladium, or tungsten or alloys thereof.

7. The method of claim 2, where said matter is a metal nanoor micro-particle, a semiconducting nano- or micro-particle, a magnetic nanoor micro-particle, a polystyrene encapsulated metal particle, a buckminsterfullerene, or a liposome-encapsulated metal particle.

8. The method of claim 2, wherein said electromagnetic field transducer functions as an antenna.

9. The method of claim 1, wherein said electromagnetic energy is radiofrequency energy.

10. The method of claim 1, wherein said radiofrequency energy has a frequency from about 100 kHz to about 40 GHz.

11. The method of claim 1, wherein said electromagnetic energy is applied magnetically.

12. The method of claim 1, wherein said electromagnetic field is inductively generated via an antenna or a series of antennae.

13. The method of claim 12, wherein said antenna(e) comprises at least one coil of electrical conductor.

14. The method of claim 13, wherein said electrical conductor is a solid wire or hollow tubing.

15. The method of claim 13, wherein said antenna(e) is a single coil antenna, a double coil antenna or a solenoid antenna.

16. The method of claim 1, wherein the reaction is a biochemical reaction.

17. The method of claim 16, wherein the biochemical reaction further comprises a pharmaceutical, a biologic, other biologically active molecules, diagnostics, or biological markers or a combination thereof.

18. The method of claim 16, wherein said biochemical reaction is enzyme catalyzed.

19. The method of claim 16, wherein said biologically active molecule has a conformational change during said biochemical reaction.

20. The method of claim 19, wherein said conformational change is denaturation, protein unfolding or protein refolding or a combination thereof.

21. The method of claim 16, wherein said biochemical reaction is a polymerase chain reaction.

22. The method of claim 16, wherein said biochemical reaction is an enzyme-linked immunosorbent assay.

23. The method of claim 1, wherein said biologically active molecule is a protein, a lipid, nucleic acids, or a carbohydrate or combination thereof.

24. The method of claim 1, wherein said biologically active molecule is in a tissue or a tissue system.

25. The method of claim 1, wherein said biologically active molecule is in vitro.

26. A method of accelerating a biochemical reaction comprising:

associating at least one electromagnetic field transducer with at least one biochemical reactant comprising said biochemical reaction;

applying radiofrequency energy to inductively generate an electromagnetic field; and

transferring energy from said electromagnetic field to said reactant(s) via said electromagnetic field transducer to increase the energy of said biochemical reactant(s) thereby accelerating the biochemical reaction.

27. The method of claim 26, wherein said biochemical reactants are biologically active molecules comprising proteins, lipids, nucleic acids, or carbohydrates or a combination thereof.

28. The method of claim 27, wherein the biochemical reaction further comprises a pharmaceutical, a biologic, other biologically active molecules, diagnostics, or biological markers or a combination thereof.

29. The method of claim 26, wherein said biochemical reactants are located in tissue or in a tissue system.

30. The method of claim 26, wherein said electromagnetic field transducer comprises matter with non-zero electrical conductivity.

31. The method of claim 30, wherein said matter is diamagnetic, paramagnetic, or ferromagnetic.

32. The method of claim 26, wherein said matter is an ionomer, a conducting polymer, an alkali metal, a transition metal, a lanthanide, or a metalloid or a combination thereof.

33. The method of claim 32, wherein said matter is colloidal or non-colloidal gold, silicon, cadmium selenide, cadmium sulfide, ruthenium, indium phosphide, indium arsenide, gallium arsenide, gold maleimide, gallium phosphide, hydroxysuccinimidyl gold, nickel-copper, nickel-palladium, palladium-cobalt, nickel-silicon, stainless steel, iron oxide, ferrite, titanium, Phynox, palladium/cobalt alloys, nitinol, titanium, titanium alloys, zirconium, gadolinium, aluminum oxide, dysprosium, cobalt alloys, nickel, gold, palladium, or tungsten or alloys thereof.

34. The method of claim 26, where said matter is a metal nano- or micro-particle, a semiconducting nano- or micro-particle, a magnetic nano- or micro-particle, a polystyrene encapsulated metal particle, a buckminsterfullerene, or a liposome-encapsulated metal particle.

35. The method of claim 26, wherein said radiofrequency energy has a frequency from about 100 kHz to 40 GHz.

36. The method of claim 26, wherein said electromagnetic field is inductively generated via an antenna or a series of antenna(e).

37. The method of claim 36, wherein said electromagnetic field transducer functions as said antenna.

38. The method of claim 36, wherein said antenna(e) comprises at least one coil of electrical conductor.

39. The method of claim 38, wherein said electrical conductor is solid wire or hollow tubing.

40. The method of claim 36, wherein said antenna(e) is a single coil antenna, a double coil antenna or a solenoid antenna.

41. A device for inductively heating biologically active molecules, comprising:

a radiofrequency power supply;

an electromagnetic field transducer; and

a means for inductively applying said radiofrequency energy to said biologically active molecules.

42. The device of claim 41, wherein said electromagnetic field transducer comprises matter with non-zero electrical conductivity.

43. The device of claim 42, wherein said matter is diamagnetic, paramagnetic, or ferromagnetic.

44. The device of claim 41, wherein said matter is an ionomer, a conducting polymer, an alkali metal, a transition metal, a lanthanide, or a metalloid or a combination thereof.

45. The device of claim 44, wherein said matter is colloidal or non-colloidal gold, silicon, cadmium selenide, cadmium sulfide, ruthenium, indium phosphide, indium arsenide, gallium arsenide, gold maleimide, gallium phosphide, hydroxysuccinimidyl gold, nickel-copper, nickel-palladium, palladium-cobalt, nickel-silicon, stainless steel, iron oxide, ferrite, titanium, Phynox, palladium/cobalt alloys, nitinol, titanium, titanium alloys, zirconium, gadolinium, aluminum oxide, dysprosium, cobalt alloys, nickel, gold, palladium, or tungsten or alloys thereof.

46. The device of claim 41, where said matter is a metal nano- or micro-particle, a semiconducting nano- or micro-particle, a magnetic nano- or micro-particle, a polystyrene encapsulated metal particle, a buckminsterfullerene, or a liposome-encapsulated metal particle.

47. The device of claim 41, wherein, wherein the power supply generates radiofrequency energy from about 100 kHz to about 40 GHz.

48. The device of claim 41, wherein said means for inductively applying radiofrequency energy is an antenna.

49. The method of claim 48, wherein said antenna is said electromagnetic field transducer.

50. The device of claim 48, wherein said antenna comprises at least one coil of electrical conductor.

51. The device of claim 50, wherein said electrical conductor is solid wire or hollow tubing.

52. The device of claim 48, wherein said antenna is a single coil antenna, a double coil antenna or a solenoid antenna.

53. The device of claim 41, wherein said biologically active molecules are proteins, lipids, nucleic acids, or carbohydrates or a combination thereof.

54. The device of claim 41, wherein said biologically active molecules are in vitro.

55. The device of claim 41, wherein said biologically active molecules are in a tissue or in a tissue system.

56. A system for inducing a biochemical reaction comprising:

a radiofrequency power supply;

a means for inductively applying radiofrequency energy to the reaction, and

a reactive composition comprising:

at least one biologically active molecule; and

an electromagnetic field transducer associated therewith.

57. The system of claim 56, wherein said biologically active molecules are proteins, lipids, nucleic acids, or carbohydrates or a combination thereof.

58. The system of claim 56, wherein said biologically active molecules are in vitro.

59. The system of claim 56, wherein said biologically active molecules are in a tissue or in a tissue system.

60. The system of claim 56, further comprising a pharmaceutical, a biologic, other biologically active molecules, diagnostics, or biological markers or a combination thereof.

61. The device of claim 56, wherein said electromagnetic field transducer comprises matter with non-zero electrical conductivity.

62. The system of claim 61, wherein said matter is diamagnetic, paramagnetic, or ferromagnetic.

63. The system of claim 61, wherein said matter is an ionomer, a conducting polymer, an alkali metal, a transition metal, a lanthanide, or a metalloid or a combination thereof.

64. The system of claim 63, wherein said matter is colloidal or non-colloidal gold, silicon, cadmium selenide, cadmium sulfide, ruthenium, indium phosphide, indium arsenide, gallium arsenide, gold maleimide, gallium phosphide, hydroxysuccinimidyl gold, nickel-copper, nickel-palladium, palladium-cobalt, nickel-silicon, stainless steel, iron oxide, ferrite, titanium, Phynox, palladium/cobalt alloys, nitinol, titanium, titanium alloys, zirconium, gadolinium, aluminum oxide, dysprosium, cobalt alloys, nickel, gold, palladium, or tungsten or alloys thereof.

65. The system of claim 61, where said matter is a metal nano- or micro-particle, a semiconducting nano- or micro-particle, a magnetic nano- or micro-particle, a polystyrene

encapsulated metal particle, a buckminsterfullerene, or a liposome-encapsulated metal particle.

66. The system of claim 56, wherein, wherein the power supply generates radiofrequency energy from about 100 kHz to about 40 GHz.

67. The system of claim 56, wherein said means for inductively applying radiofrequency energy is an antenna.

68. The method of claim 67, wherein said antenna is said electromagnetic field transducer.

69. The system of claim 67, wherein said antenna comprises at least one coil of electrical conductor.

70. The device of claim 69, wherein said electrical conductor is solid wire or hollow tubing.

71. The device of claim 67, wherein said antenna is a single coil antenna, a double coil antenna or a solenoid antenna.

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