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(74) Agents: **FALLON, Steven, P.** et al.; Greer, Burns & Crain, LTD., 300 South Wacker Drive, Suite 2500, Chicago, Illinois 60606 (US).

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(71) Applicant (for all designated States except US): **UNIVERSITY OF LOUISVILLE RESEARCH FOUNDATION, INC.** [US/US]; 2301 South Third Street, Jouett Hall, Suit LL02, Louisville, Kentucky 40208 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **KANG, Kyung, Aih** [US/US]; 9023 Green Garden Court, Louisville, Kentucky 40220 (US). **SHI, Donglu** [US/US]; 493 Rhodes Hall, University Of Cincinnati, Cincinnati, Ohio 45221-0012 (US).

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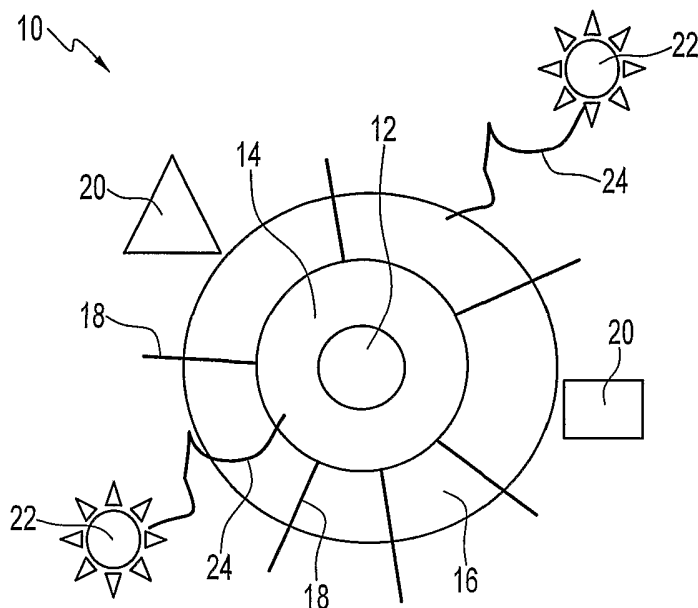
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(54) Title: **TARGET SPECIFIC NANOPARTICLES FOR ENHANCING OPTICAL CONTRAST ENHANCEMENT AND INDUCING TARGET-SPECIFIC HYPERTHERMIA**



(57) Abstract: A particle for introduction into a biological or physiological system for enhancing optical contrast between target cells and surrounding tissue, and for inducing cell death in the target cells while minimizing effects on surrounding healthy tissue, where the particle includes a magnetic core, a metal nanolayer surrounding the magnetic core and a surfactant layer surrounding the metal nanolayer. Bound to the metal nanolayer is at least one specificity biomolecule having an affinity for the target cells is bound to the metal nanolayer and a therapeutic agent. The particle may be introduced into a biological or physiological system, and exposed to an electromagnetic field to induce hyperthermia in the target cells.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TARGET SPECIFIC NANOPARTICLES FOR ENHANCING OPTICAL CONTRAST ENHANCEMENT AND INDUCING TARGET-SPECIFIC HYPERTHERMIA

TECHNICAL FIELD

A field of the invention is optical enhancement. Another field of the invention is disease detection and treatment.

BACKGROUND ART

Improving cancer prevention, detection and treatment are all important goals in the fights against cancer, where enhanced methods of detection are needed for earlier, more accurate and less invasive detection, as are effective yet precise treatments of the disease.

The ultimate goal of cancer therapy is to destroy cancer cells while minimizing damage to the normal, healthy cells. This is frequently problematic in that many therapeutic agents used to combat cancer are also toxic to the normal, healthy cells, and cause harmful side effects in patients. Targeting the cancer cells while leaving the healthy cells unaffected has presented a difficult challenge in cancer treatment.

Current cancer detection methods present additional obstacles. For example, X-ray mammography is a method commonly used for breast cancer detection, but X-ray mammography has low sensitivity for younger women (<40 years) due to their dense breast tissue. If a tumor is suspected from an X-ray mammogram, invasive removal of tissue is needed from the suspected region for a biopsy, which often turns out to be benign tumors. This process usually generates both physical and mental suffering for patients undergoing diagnosis via X-ray mammogram. Similarly, optical mammography, which uses near infrared light, is a relatively new detection approach, especially for younger women. Tumors generate blood vessels, and therefore tend to have more blood and more hemoglobin, which is a natural but strong chromophore in tissue. This results in high absorption of near infrared light. Optical mammography methods can detect tumors as small as 0.5 mm.

While there are a number of benefits to this technique, tumors that are particularly small or deep seated within the breast have weak optical contrast and are still difficult to detect.

DISCLOSURE OF THE INVENTION

Embodiments of the invention include a particle for introduction into a biological or physiological system for enhancing optical contrast between target cells and surrounding tissue, and for inducing cell death in the target cells while minimizing effects on surrounding healthy tissue. The particle includes a magnetic core, and may also include a metal nanolayer surrounding the magnetic core and a surfactant layer surrounding the metal nanolayer. The particle may further include at least one specificity biomolecule having an affinity for the target cells, where the specificity biomolecule bound to the metal nanolayer. A therapeutic agent may also be bound to the metal nanolayer to induce cell death.

Other embodiments include a non-invasive method of simultaneously locating and destroying target cells. A cytotoxic particle is formed by providing a magnetic nanoparticle, surrounding the magnetic nanoparticle with a metal nanolayer having high surface plasmon density, and surrounding the metal nanolayer with a surfactant layer. At least one therapeutic agent is bound to the metal nanolayer, as is at least one specificity biomolecule. The cytotoxic particle is then introduced to a biological or physiological system, where optical contrast is observed to determine a location of the target cells. The target cells may then be exposed to an alternating electromagnetic field to induce hyperthermia via the magnetic core in the target cells.

BRIEF DESCRIPTION OF THE DRAWING

FIGURE 1 is a schematic diagram of a nano-entity according to a preferred embodiment of the invention;

FIG. 2 is a schematic diagram illustrating an exemplary linking method of a fluorophore to a nanogold particle;

FIGs. 3a and 3b are schematic diagrams illustrating (a) near infrared measurement for the fluorescent contrast by nanogold particle linked ICG and

absorption contrast by the magnetic nanoparticles in an animal model, and (b) alternating electromagnetic field application for hyperthermia;

FIG. 4 is a schematic diagram illustrating Basic principle of an induction heater;

5 FIGs. 5a and 5b are schematic diagrams illustrating (a) a hand-held, pancake shaped induction heater; and (b) double pancake shaped induction heater;

FIG. 6 is a schematic diagram illustrating Experimental Model Study: optical contrast measurement by near infrared optical mammography in an experimental study;

10 FIGs. 7a and 7b are graphs illustrating cypate fluorescence enhancement in (a) free form and (b) in surface immobilized cypate via protein A on various nanogold particles;

FIGs. 8a and 8b are graphs illustrating cypate fluorescence enhancement in a tumor model in (a) a mixture of cypate and protein A inked nanogold particles in free form and (b) in surface immobilized form by various nanogold particles in a breast tissue like model when the tumor model is placed at 1 cm deep;

FIG. 9 is a graph illustrating an effect of particle size on heating performance of iron oxide nanoparticles by an alternating electromagnetic field;

20 FIGs. 10a and 10b are a schematic diagram illustrating an experimental set-up for application of a (a) coil and (b) pancake shaped induction heaters on a breast model;

FIGs. 11a and 11b are schematic diagrams (front view) illustrating (a) single and (b) double pancake shaped induction heater coils and the simulation results of their magnetic field distribution; and

25 FIG. 12 is a graph illustrating heating of various depths of tumor models containing of 1wt% Fe_3O_4 nanoparticles in the air or in the meat (chicken breast tissue).

BEST MODE FOR CARRYING OUT THE INVENTION

30 Two problems frequently arise in managing disease, particularly cancer. First, localization (detection) of the disease site is an imperfect art, especially where

the diseased tissue is small and deeply seated, obscuring the tissue from optical imaging detection. Second, targeting the diseased tissue with specificity while leaving healthy tissue unaffected also presents some difficulty to practitioners seeking to induce toxicity to diseased tissue, either via hyperthermia or other therapeutic agent.

5 It would be particularly advantageous to have a single nano-entity and methods of using that nano-entity whereby delivery to a tumor or other disease site is specific with minimal distribution to normal tissue or excretion organs, and provides a guide for thermal energy to the tumor to non-invasively induce death of the diseased tissue, via one or more of hyperthermia or other therapeutic agent.

10 It is known that increasing cell temperature to a particular level results in cell death. More particularly, when, for example, human tissue is heated past a set temperature, such as 45° C, various enzymes within the tissue are deactivated, leading to a slow cell death. Thus, by selectively heating cancer cells without similarly heating the surrounding healthy tissue, cancer cells may be selectively destroyed while
15 leaving healthy cells unaffected, thereby rendering a powerful tool in the precise and effective treatment of cancer.

Separately, electrically conductive materials, such as magnetic nanoparticles, generate heat in an alternating electromagnetic field. At an appropriate frequency, an induction heater can heat magnetic nanoparticles to a desired
20 temperature without heating tissue. Thus, by introducing magnetic nanoparticles to target cells, such as a tumor site, the target cells may be selectively heated to induce toxic hyperthermia of the target cells, while non-target cells remain unaffected.

Determination of the location of the target cells is helpful to the precise introduction of nanoparticles for the purposes of destroying the target cells. Optical
25 imaging methods are frequently used for this purpose. For example, near infrared imaging methods are effective in cancer detection, such as breast cancer detection, even when the tumors are not amenable to X-ray detection. However, when a tumor is very small and deeply seated, enhancement of contrast for imaging purposes is especially advantageous.

Fluorophores are known to be good contrast agents, and a few of these fluorophores are known to be suitable for use with human subjects, such as indocyanine green (ICG) or Cytopate, with ICG also being used for its high near infrared absorption.

Metals having a high surface plasma density are also known to be good contrast agents. For example, gold is a particularly good contrast agent, and owing to its chemically inert nature, is compatible with human use. Additionally, the optical resonance wavelength for gold may be easily tuned to a broad range, especially to the near infrared range, by controlling sizes and shapes of gold particles. Thus, nano-sized gold particles, or "nanogold particles" ("NGPs"), as well as relatively thin gold surfaces, may also enhance fluorescence, and may advantageously be used to link other biomolecules. For example, therapeutic peptides, proteins, or other polymers may be linked, either for adding target specificity to a nanoparticle or for delivering a tumor-cytotoxic payload to a target site.

Embodiments of the invention include selectively introducing magnetic nanoparticles to a target tissue and inducing tissue hyperthermia by heating. When magnetic nanoparticles are introduced to target tissue, such as a tumor site, the nanoparticles preferentially accumulate in and around the tumor due to the unorganized nature of tumor vasculature. Cells of the target tissue may then be heated until they have reached a predetermined temperature calculated to result in cell death.

Other embodiments of the invention include selectively introducing image contrast enhancing nanoparticles to a target tissue to enhance optical contrast for diagnostic and other purposes. The nanoparticles preferentially accumulate at a tumor site, and function as effective optical contrast agents for detection of even a small and deeply seated tumor, if present.

Yet another embodiment of the invention includes preparing magnetic nanoparticles to target particular cells or other analytes, such as tumor cells, by providing tumor anti-receptors with the nanoparticles.

Still other embodiments include introducing self-temperature-controlling magnetic nanoparticles to a target tissue and inducing tissue hyperthermia using via induction heating. It is known that some magnetic materials lose their magnetic

properties when they are heated to a certain temperature, which is known as the “Curie temperature.” The self-temperature-controlling magnetic nanoparticles are configured to exhibit a Curie temperature that is either at or below the desired temperature for inducing toxic hyperthermia in the cells, such that the cells exposed to the self-temperature-controlling nanoparticles are prevented from being heated above the Curie temperature, thereby maximizing efficacy of the toxic hyperthermia and minimizing damage to surrounding tissue.

Yet other embodiments of the invention provide a target site specific, multi-functional nano-entity that provides a high optical contrast for target site localization (detection), which may also serve as a guide for target site specific hyperthermia with minimal accumulation in unintended areas. More particularly, magnetic particles are coated with a metal nanolayer having a high surface plasmon density to enhance fluorescence contrast properties. These particles, when delivered specifically to a target site with minimal distribution to normal tissue or excretion organs, may guide thermal energy specifically to the target site by non-invasive application of an alternating electromagnetic field.

While it is contemplated that embodiments of the invention may be used in connection with diagnosis and treatment of a wide array of cancers and other diseases, for purposes of illustration, embodiments will be shown and described in connection with breast cancer. Breast cancer remains the most common form of cancer among women, with an estimated 211,240 new cases and 40,410 deaths in United States in 2005. Although the death rate has been reduced by the introduction of breast screening mammography and adjuvant therapies, more accurate, early detection and efficacious treatment modalities would be advantageous.

Turning to FIG. 1, a preferred embodiment includes a cancer specific, multi-functional nano-entity that provides high optical contrast for tumor localization (detection), and which may also serve as a guide for tumor specific hyperthermia with minimal accumulation in the excretion organs. The nano-entity may then be introduced into a human body or other system wherein the nano-entity will accumulate at a tumor site and may optionally be used to induce hyperthermia in cancer cells at the tissue site.

More particularly, a nano-entity, designated generally at 10, is illustrated in FIG. 1. The nano-entity 10 includes a core 12 that is preferably a magnetic nanoparticle, where the magnetic nanoparticle is enclosed in a metal nanolayer 14 that enhances optical/fluorescence contrast for tumor localization. While it is contemplated that the core 12 may include one of a variety of magnetic nanoparticles to suit individual applications, consideration may be given to several factors when determining which nanoparticles to introduce to the target tissue, such as size, magnetism/ferromagnetism and biocompatibility, to name a few.

Magnetic nanoparticles may be used to deliver heat to destroy the tumor site via hyperthermia under an alternating electromagnetic (AEM) field without heating the medium surrounding them. While it is contemplated that numerous magnetic nanoparticles may be used to deliver heat, preferred nanoparticles are stable and non-flammable. For example, preferred particles include iron oxide nanoparticles (e.g., Fe_2O_3 or Fe_3O_4), insofar as the Food and Drug Administration (FDA) has already approved iron oxide based as MRI contrast agents for human use, indicating that the toxicity of iron oxide nanoparticles is likely minimal. By nature, materials composed of iron oxide nanoparticles are dark and, therefore, amenable for use as optical contrast agents. The magnetic nanoparticles may be used alone to induce hyperthermia, or may be included in the preferred nano-entity 10 as the magnetic core 12.

Additionally, ferromagnetism is a phenomenon by which a material can exhibit a spontaneous magnetization, and forms the basis for all permanent magnets. All ferromagnets have a maximum temperature where the ferromagnetic property disappears as a result of thermal agitation. This temperature is called the Curie temperature. By manipulating the composition of alloys exhibiting ferromagnetism, as well as manipulating the size of alloy nanoparticle, the Curie temperature can be customized to suit individual applications. By manipulating alloy composition and nanoparticle size, it is possible to obtain magnetic nanoparticles with the Curie temperature of between approximately 40 - 50 °C, which is the preferred range according to the instant embodiment, whereby hyperthermia may be safely induced while simultaneously minimizing the complexity of an AEM instrument used

therewith. Alloys of iron/nickel and nickel/copper tend to have Curie temperatures in the preferred range, and are therefore preferred alloys for the instant embodiment. Thus, these alloys materials may additionally be included in the preferred nano-entity as the core 12.

5 With respect to the metal nanolayer 14, it is preferred that the metal nanolayer include a metal having high surface plasmon field strength, which also appear to be those metals having electron affinity. For example, gold, copper, silver and platinum may be used as the metal nanolayer 14.

 While the invention contemplates a variety of constituent metals for use
10 with the metal nanolayer 14, a nanogold layer (NGL) is preferred owing to the chemical inertness and non-toxicity of gold to biological and physiological systems. NGLs also have a strong plasmon field around them, which can be used for rerouting electrons from other molecules. The plasmon field may be used to enhance the fluorescence of fluorophores, for example. NGLs also exhibit a very high near
15 infrared absorption and scattering, thereby providing advantages as a contrast agent for optical tools. NGLs are also advantageous in that they can be easily tuned to a broad range, especially to the near infrared range, by controlling the size and shape of the particles. Additionally, gold layers are amenable to immobilizing various biomolecules.

20 The metal nanolayer 14 can be created by first adding additional NaBH_4 aqueous solution to core 12 nanoparticle solutions, which will expand the micelle to make room for the metal nanolayer. This is followed by an addition of an aqueous solution of HAuCl_4 or other metal, forming a gold or other metal nanolayer. In one preferred embodiment, the solution is prepared in the following steps: 2 volume 3.5
25 mol/l $(\text{NH}_4)_2\text{SO}_4$ solution and 1 volume 25% $\text{NH}_3\text{H}_2\text{O}$ are added into 2 volume 0.14 mol/l AuNO_3 solution, and then 2 volume 0.35mol/l CoSO_4 is added. Distilled water is preferably used to prepare the solution; otherwise the ions in the water would combine the Au^+ and produce insoluble precipitation. To accelerate the coating process, 0.15 g/l KBr is used as an accelerator. In the preferred embodiment, the Co
30 particles formed will also act as an active substance as well as an efficient catalytic

surface for the deposition of gold ions. The complete gold nanolayer 14 can be easily formed by this solution coating method.

In addition to the magnetic core 12 and metal nanolayer 14 surrounding the magnetic core, the nano-entity 10 may also include additional features to enhance optical imaging properties as well as features to enhance efficacy of disease treatment. These features may include a surfactant layer 16 that preferably includes hydrophilic polymers bound to the surface of the nano-entity 10 to promote biocompatibility of the nano-entity. Additionally, the nano-entity may include target specific anti-receptors to precisely guide the nano-entity to the correct target site, as well as a fluorophore to enhance fluorescence.

The surfactant layer 16 is preferably disposed over the metal nanolayer 14. Conventional plasma or chemical treatment has been currently used in surface and interface engineering for improving adhesion, hydrophobicity, hydrophilicity, printability, corrosion resistance, selectivity, or for surface etching or cleaning. The main principle of the plasma is that ionized and excited molecules created by an electrical field bombard and react on the surface of a substrate. The activated molecules may etch, sputter, or deposit on the substrate surface. As a result, the surface properties of substrates can be modified and thin film can be deposited on almost any substrates, including metal nanoparticles. It is an environmentally benign process at a room temperature.

It is contemplated that, where provided, the composition of the surfactant layer 16 may vary to suit individual applications, and may be selected from a vast number of biocompatible polymers. By way of example only, several exemplary polymers suitable for use as the surfactant layer 16 may include an acrylic acid coating, acetylene, pyrrole, and others.

Generally, nanoparticles are difficult to disperse in the plasma polymerization coating process due to aggregation and large surface area per unit mass. In the plasma thin film coating process, the surface of the nanoparticles are exposed to the plasma. The unexposed regions of the powder particles are hardly modified. A fluidized bed reactor is an ideal tool for gas-particle reactions due to the

intensive mass and heat transfer between the two phases, short reaction time, and flat temperature profile. Therefore, the combination of plasma polymerization and the fluidized bed process represents an innovative approach for near-room temperature surface modification of particles. This is particularly important for biomedical products to retain its bioactivities.

By way of example only, one exemplary surfactant layer 16 includes an acrylic acid coating, which enables nanoparticles to be soluble in water. By fine-tuning the deposition conditions, it is possible to retain a high concentration of carboxyl groups in the films. A tumbler reactor can mechanically agitate particles inside a horizontal vessel by means of a rotating shaft with vanes and the other reactor distributes the particles in the plasma by fluidizing them. Nanoparticles can be easily treated in the fluidized bed reactor because they are spherical, light weight and flow easily.

Turning again to FIG. 1, the nano-entity 10 preferably includes one or more target specific anti-receptors 18 to impart target specificity to the nano-entity such that it preferentially binds and/or accumulates at the binding sites of the target cells. The target specific anti-receptors 18 may be provided in combination with a therapeutic agent 20, such that the anti-receptors bind the target cells and the therapeutic agent provides a cytotoxic payload.

While it is contemplated that a virtually limitless number of anti-receptors may be provided to suit individual applications, and that a corresponding number of therapeutic agents may also be provided to address individual diseases, several exemplary anti-receptors and therapeutic agents will be discussed herein for purposes of illustration.

Exemplary anti-receptors include luteinizing hormone releasing hormone (LHRH), monoclonal antibodies, and aptomers, to name a few. Using LHRH as a specific example, breast tumor specific targeting may include LHRH, which is a hormone with a peptide chain of 10 amino acids (approximately 1,400 Daltons) that controls sex hormones in men and women. Under normal physiological conditions, LHRH is synthesized in the hypothalamus. It is secreted in the median

eminence of the pituitary, where it enters the capillary plexus and is transferred via the long portal vessel to the gonadotrophic cells of the adenohypophysis. LHRH stimulates the synthesis and release of two pituitary gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), both of which are essential
5 for the regulation of normal reproductive functions in most mammals.

However, the receptors for LHRH are over-expressed in most of breast, ovarian and prostate cancer cells. LHRH receptors are not expressed detectably in normal organs. Therefore, the differential receptor expression of LHRH is a useful tool in selectively targeting breast, ovarian and prostate cancer cells. The use of
10 LHRH peptides as a targeting moiety substantially enhances the uptake of the anti-cancer drugs or magnetic nanoparticles (Fe_3O_4 ; 20 nm) in a tumor with much less accumulation in liver or kidney as compared to the magnetic nanoparticles alone. Thus, LHRH peptides provide an exemplary anti-receptor 18 that may be used with the nano-entity 10 according to the preferred embodiment.

15 Contrast enhancing agents are also preferably provided with the nano-entity. Contrast enhancing agents include fluorophores 22, which may be additionally coupled to a spacer 24 to maximize fluorescence enhancement by maintaining an optimal distance between the fluorophore and the gold nanolayer 14.

While it is contemplated that numerous fluorophores 22 may be used
20 with various embodiments of the invention, compatibility of the fluorophore with biological and physiological systems is preferred. Thus, a preferred exemplary fluorophore is Indocyanine green (ICG), which is an FDA approved, strong near infrared absorber. Another preferred fluorophore is cypate, which is a derivative of ICG. Still other exemplary fluorophores include rhodamine and fluorescein.

25 The metal nanolayer 14 can increase fluorescence emission yield of the fluorophore 22 by eliminating the free electrons involved in self-quenching, via the strong plasmon field attached on the surface of the metal nanolayer. This enhancement process is dependent on the nanoparticle size as the distance between the fluorophore molecules and the metal nanolayer 14.

Cypate (M.W. = 705) has a carboxylic group, which allows it to be linked onto the metal nanolayer 14 and magnetic core 12. As illustrated in FIG. 2, to manipulate the distance between the fluorophore 22 (cypate) more precisely, the cypate molecules were linked onto various metal nanolayers 14 via the protein spacer 24. Protein A (PA) and streptavidin (SA) were reacted through the reaction between the carboxylic group in cypate and the amine group in the protein. The spacer lengths were estimated by computer simulation and they were approximately 1 and 3 nm for PA and SA, respectively.

The preferred nano-entity 10, as illustrated in FIG. 1, includes the metal nanolayer 14 surrounding the magnetic nanoparticle core 12. The hydrophilic surfactant layer 16 preferably surrounds the metal nanolayer 14 and core 12 to enhance biocompatibility. While the nano-entity will naturally accumulate in tumor tissue owing to the unorganized vasculature of the tumor tissue, specificity is preferably enhanced by providing anti-receptors 18 to the target cell or other target analyte.

Optical imaging is enhanced by the metal nanolayer 14 surrounding the magnetic core 12, but may further be enhanced by providing the fluorophore 22 spaced at an appropriate distance from the metal nanolayer, such as by the spacer 24. While induced hyperthermia is effective at destroying the target cells or other analytes, the preferred nano-entity 10 further includes one or more therapeutic agents 20, such as doxorubicin, taxol, tamoxifen and others.

Various embodiments of the invention include detection and treatment of disease using the nano-entity 10. Embodiments are especially advantageous in that detection and treatment are seamless, because it is possible to use the same nano-entity 10, once introduced to the target system, for both detection and treatment, without introducing additional agents into the system.

The nano-entity 10 is introduced into a biological or physiological system, such as a human body, via one of a plurality of ways. While the invention contemplates numerous methods of introducing the nano-entity 10 to the body, preferred methods include injection, intravenously, intratumorally, via suppository or transdermally.

Detection of the target site is accomplished by detection of an accumulation of the nano-entity 10 at the target site, owing both to the natural accumulation of the nano-entity at unorganized vasculature as well as the binding of the anti-receptors 18 of the nano-entity to a particular cell or tissue. Optical contrast may be measured by near infrared spectroscopy (NIR). FIGs. 3a and 3b are schematic diagrams illustrating near infrared measurement for the fluorescent contrast by nanogold particle linked ICG and absorption contrast by the magnetic nanoparticles in an animal model. Included are a near infrared source probe 25a and a detector probe 25b. A tumor 26 is the target of the source probe 25a and detector probe 25b

Advantageously, where the nano-entity 10 includes both optical image enhancing agents as well as a therapeutic agent 20, detection and treatment may at least partially coincide, without additional steps being taken. Additionally, treatment may further include induced hyperthermia by exposing the target site and the nano-entity accumulated at the tumor site to an alternating electromagnetic field. Because the preferred nano-entity is target specific, the nano-entity will have predominantly accumulated only at the target site, thereby permitting hyperthermia only at the target site while leaving the surrounding areas unaffected.

For decades, alternation electromagnetic heaters, which are usually called induction heaters, have been used for characterizing thermal properties of various metals. Turning to FIG. 4, the basic components of an induction heating system, generally at 28, are an AC power supply (not shown) and an induction coil 30. The power supply sends alternating current, represented by arrows 31a, through the coil 30, inducing an alternating current, represented by arrows 31b, generating a magnetic field 32. Many industries requiring the melting metal bars have had this type of heater for several decades at frequencies lower than 0.5 MHz and capitalize on the property of induction heating. Similar to the principles of the induction heater 28, but at frequencies greater than 1 GHz, is a microwave using dipole heating as the heating mechanism.

Using similar instruments, but at relatively lower frequencies, such as at less than 1 MHz, a heating instrument may be designed to heat magnetic nanoparticles

without generating induction heating or dipole heating (tissue heating). The hysteresis caused by magnetic properties of the magnetic nanoparticles generates a certain amount of heat for the magnetic nanoparticles. This method of heating is safe insofar as the preferred magnetic nanoparticle core 12, which preferably includes
5 nanoparticles of γ -Fe₂O₃ or Fe₃O₄, have been approved by the FDA for MRI contrast agents. Additionally, the heating instrument is relatively inexpensive, clean and applies localized heat for magnetic particles without requiring physical contact with the material. The shape of the coil associated with the heating instrument may be cylindrical, flat, two-dimensional, or circular (pancake) types, which makes the
10 application on the body surface easier.

The invention contemplates that a variety of alternating electromagnetic field devices may be used for hyperthermia, and such devices may be configured to suit individual applications. For purposes of illustration only, FIGs. 5a and 5b illustrate an exemplary hand-held, pancake shaped induction heater 34a and a double
15 pancake shaped induction heater 34b, respectively.

Preferred electromagnetic field instruments 34a, 34b are portable, preferably hand-held, induction heaters (shown also in FIG. 3b). A coil 36 may be configured to be generally cylindrical or pancake in shape, with the pancake shape being preferred insofar as the pancake shape is well suited and may be applied
20 conveniently and non-invasively on the surface of breast tissue, for example. The coil 36 is water cooled and is configured such that, at frequencies below 500 KHz, a magnetic field does not heat any tissue components. Also, to efficiently apply the magnetic field to the tumor site, a size of the induction heater coil 36 may be optimized to have a relatively small size, for example less than 10 cm. A control
25 panel 38 is also preferably provided to control the temperature of the tumor site, as well as a power source 39, such as an AEM generator. Thus, the preferred electromagnetic field instrument is a safe, user-friendly, hand-held unit exhibiting portability and ease of use.

Various embodiments may also include a double pancake type,
30 induction heater configuration for deeply seated tumors. System parameters such as input power (P), frequency of the alternating electromagnetic field (f), number of the

coil turns (n), and the diameter of the coil (d) are optimally provided to promote hyperthermia, which includes considering the penetration depth of the electromagnetic field and SAR absorbed by the nano-entity 10. Once the heating rate and the penetration depth is known for a particular induction geometry, then, to keep the particles temperature constant (40~50°C), the power supply need to be automatically turned on-off at a constant time interval.

Experimental Results and Data

Optical contrast by NGPs is enhanced. Near infrared (NIR) has been recently studied extensively to non-invasively detect breast tumors. Dense vasculature around a tumor usually creates high optical contrast created by higher concentrations of hemoglobin for tumor in NIR region. Optical contrast agents, such as fluorophores or NIR absorbers can frequently help the cancer detectability.

Photon absorption properties of various sized (5~250 nm) NGPs demonstrate that NGPs absorb photons in near infrared well. NGPs at a size 150 nm showed a high absorption (optical density of 0.3 at concentration of 0.01%) at 780 nm, which is the wavelength frequently used for optical mammography.

To test the optical contrast produced by the NGPs, optically, breast-tissue like models (0.03 and 8 cm⁻¹ for absorption and scattering coefficients, respectively; FIG. 6) were made, using agar, skimmed milk, and India ink in water to have the optical properties. The breast tissue model 27 dimension (24x14 cm) was based on the compressed breast tissue between two mammogram plates, and the model thickness was 5 cm (the average compressed breast is 4.7 cm). They were then embedded with a nanoparticle containing tumor model 26, made with an empty Vitamin E capsule shell filled with either nanogold particles (150 nm) at 0.01% or gold coated Fe₃O₄ particles (diameter, 10~20 nm) at 1% was placed 1 cm deep from the surface of the breast model.

For the transmittance measurements, the NGP-containing tumor model showed the maximum contrast of 3.5 dB with only 0.01% particle concentration. Gold coated, Fe₃O₄ containing tumor model, at a concentration of 0.1%, showed the maximum contrast of 3 dB.

Indocyanine green (ICG) is an FDA approved, strong NIR absorber and also a fluorophore. To test the effect of ICG as an absorption contrast agent, a heterogeneity containing ICG at a concentration of 32 μM , i.e., ICG filled in an empty vitamin E capsule shell, was embedded at various depths from the model surface. Then the model surface was scanned at 780 nm, at 3 cm source-detector (S-D) separation. The measurement was taken in a transmission mode, since for the absorbing heterogeneity, it provides better localization than reflectance mode.

The light intensity (ΔMR) values at the modulation frequency of 0.1 GHz from the area (5.0 x 5.0 cm) around the heterogeneity, located at a depth of (a) 1.0, (b) 1.5, (c) 2.0, or (d) 2.5 (the middle of the breast thickness) was observed. For the heterogeneity located at the 1.0, 1.5, 2.0, and 2.5 cm depth, the maximum ΔMR decrease was 5, 4, 3.4, and 1.5 dB, respectively.

As stated, ICG is one of a few fluorophores known to be compatible for use with humans, though it is anticipated that more may be available in the future. It emits light at 830 nm wavelength when excited at 780 nm light. Measurements for fluorescence were performed for the same model used for the absorption study above, but using a long pass optical filter for the fluorescence detection. Changes of the light intensity (ΔMR), when the measurements were done in reflectance at a source-detection (S-D) separation of 2 cm were observed. The maximum ΔMR increase was 22, 12, 8, and 3.3 dB, for 1, 1.5, 2, and 2.5 cm, respectively. The fluorescent contrast was decreased to 3.3 dB as the heterogeneity moved to 2.5 cm deep in the breast model.

Cypate (M.W. = 705) is an ICG derivative with carboxylic groups. Cypate has a low quantum yield, as ICG, approximately 0.012 in water or saline and 0.032 in plasma, which limits its effectiveness in biomedical research. Cypate has a carboxylic group, which allows it to be linked onto the nanogold particle, while ICG is difficult to react with other molecules. The possibility of its fluorescence enhancement by various nanogold particles was studied. When NGPs are linked to ICG, an increase is observed in the fluorescence emission yield of a fluorophore by eliminating the free electrons involved in self-quenching, via their strong plasmon

field attached on the surface. This enhancement process has been proved to be dependent on the nanoparticle size and the distance between the fluorophore molecules and the NGP.

To manipulate the distance more precisely, the cypate molecules were
5 linked onto various NGPs via a protein spacer. Protein A (PA) and streptavidin (SA) were reacted through the reaction between the carboxylic group in cypate and the amine group in the protein (FIG. 2). The spacer lengths were estimated by computer simulation and they were approximately 1 and 3 nm for PA and SA, respectively.

5 nm or 10 nm NGPs coated with protein A (approximately, 1 nm) or
10 streptavidin (approximately, 3 nm) were tested and the fluorescence signal intensities for cypate only, nanogold particle linked cypate, and the mixture of cypate and nanogold particles without physical binding were measured at a constant cypate concentration of 30 μ M.

FIGs. 7a and 7b show the fluorescence enhancement levels of (a) free
15 cypate and (b) surface immobilized cypate by various nanogold particles, compared to the signal of cypate only. Experimental conditions included a cypate concentration of 30 μ M and NGP, nanogold particle; PA, protein A (~ 1 nm); SA, streptavidin (~ 3 nm); 5 nm and 10 nm sized NGPs. 1 nm surfactant linked 10 nm NGP presented an optimal enhancement of ~2000%. FIG. 7(b) shows the enhancement when cypate is
20 physically linked to the particles. The NGP at sizes 5 nm and 10 nm were linked to either PA or SA. For all three cases, the fluorescence of the surface immobilized cypate was enhanced by 4~5 orders of magnitude of the signals of cypate only. When the distance between the cypate and the nanogold particle was kept constant, 5 nm nanogold particles showed approximately three times of enhancement of that by 10
25 nm. The effect of the distance on the cypate enhancements was studied when the nanogold particle size was fixed. For 10 nm sized nanogold particle linked cypates with two kinds of spacers used, PA and SA, the distance created by SA (~3 nm) has provided about 100% higher enhancement than that by PA (1 nm) separation.

As a next step, the fluorescence contrasts by cypate and cypate linked
30 NGPs with spacers of PA and SA and the free mixture of the cypate and NGPs were

measured in the breast-tissue like experimental model, shown in FIG. 6. To test the effect of cypate and cypate linked NGPs as fluorescence contrast agents, a heterogeneity containing these contrast agents at a concentration of 5 μ M. The mixture of the contrast agents and the ingredient of the breast model were filled in an empty vitamin E capsule shell at a dimension of 1.2 x 0.7 x 0.5 cm and the capsule was embedded at 1cm depth from the model surface. Then the model surface was scanned using NIR-time resolved spectroscopy (TRS) in reflectance with 2 cm source and detector (S/D) separation (FIGs. 8 and 8b). Cypate at a concentration of 5 μ M was enough to get fluorescence signal. Cypate linked nanogold particles at a size 5 and 10 nm, via PA and SA, gave very similar enhancement, i.e., approximately 50~100 % higher fluorescence contrast than cypate alone. However, the free mixture of cypate and nanogold particles gave little enhancement on the cypate fluorescence signal.

For the selection of nanoparticles, gold nanoparticles at the size range of 0 ~ 1000 nm, were first selected because of their non-toxic and inert nature to bio-/physiological systems and also easy access for the conjugation to tumor specific antibodies or other therapeutic agents on their surfaces. The particle size of 1000 nm was considered to be the maximum because particles larger than this may not be easily delivered through small capillaries (~3000 nm). The heating of the gold nanoparticles was minimal. The heating effect of metal with high resistivity, in this case titanium, whose resistivity (42 $\mu\Omega$ -cm; ~10 nm) is approximately 18 times higher than gold, was tested at the same experimental conditions. Titanium particles were not heated, either, implying that the mechanism of the heating is not by induction or electrical resistivity.

To find the AC frequency of AEM field that can be used for nanoparticle mediated hyperthermia but not heating normal tissue, the heating of normal tissue and tissue components were tested at various frequencies. The samples (4 ml, each) tested were distilled water, 0.9% NaCl solution, hemoglobin solutions (bovine) at a concentration of 0.14 g/ml-water (normal tissue) and 0.56 g/ml-water (the usual concentration at or around the tumor), and ground beef. The sample was placed in a glass tube and they were placed in a cylindrical shape AEM coil at

frequencies of 0.45, 5.4, and 9.2 MHz, at 5 KW for 2 minutes (Table 1). At all frequencies, water was not heated. 0.9% of NaCl solution and the ground beef were easily heated at 5.4 and 9.2 MHz. Hemoglobin did not get heated at either 0.45 or 5.4 MHz. The frequency of 0.45 MHz or lower was, therefore, selected for further studies.

Table 1

Samples	Concentration	Δ Temp ($^{\circ}$ C)		
		0.45 MHz	5.4 MHz	9.2 MHz
Distilled H ₂ O	-	2.7	3.0	2.9
Ground beef	-	3.2	5.9	8.3
Hemoglobin	0.14 g/ml	3.2	1.8	-
	0.56 g/ml	3.6	2.5	-
NaCl solution	0.9%	3.1	12.7	11.2

When a magnetic material, including nano-magnetic particles, is placed in an AC EM field, the heating effect is greatly enhanced, possibly due to the hysteresis loss, Neel relaxation, or/and Brownian relaxation. One of the frequently tested magnetic particles is Fe₃O₄ and the effect of nanoparticle size on heating performance was studied at 0.45 MHz and 5 KW. FIG. 9 shows the temperature increase in the samples (in agar) containing the iron oxide nanoparticles at various sizes, after exposing in the alternative electromagnetic field (AEM) field for 2 minutes. The concentrations of iron oxide in the samples were 0.1 ~ 1wt%. The samples were heated at a 0.45 MHz frequency and 5 KW power for 2 minutes. The concentrations of iron oxide nanoparticle in the sample were from 0.1 to 1wt%.

For all particles the heating was linearly proportional to the particle concentration in the sample. For the samples containing Fe₃O₄ at 10-20 nm or 20-30 nm, the temperature increases were approximately at a rate of 35 $^{\circ}$ C/1wt% of particles. With Fe₃O₄ particles at 40-60 nm, however, it was 9 $^{\circ}$ C/1wt%, indicating the heating capability of the iron oxide nanoparticles depend on their particle sizes. For the sample containing 20-30 nm size of γ -Fe₂O₃, the rate of temperature increase was 30 $^{\circ}$ C/1wt%, which is very close to the Fe₃O₄ particles at the range of 10~30 nm. For the Feridex I.V.®, which is 5 nm Fe₃O₄ based MRI contrast agent, the rate of temperature increase was 3 $^{\circ}$ C/1wt%. Although the heating rate is rather slow,

considering it is FDA approved and only 2 minutes of heating increases 3°C, extending the AEM application time can achieve the desired temperature.

Some of surface treated Fe₃O₄ particles were prepared. The particles at the size of 10~20 nm were chemically coated with gold, and also coated with acrylic acid for better hydrophilicity and biocompatibility by plasma deposition technique. The particles were dispersed in 4 ml of agar solution by a sonicator at a concentration of 0.1 or 1%. Once the agar containing Fe₃O₄ particles was solidified, AEM field was applied to them for 2 minutes, at 5 KW (Table 2). The temperature increase for the sample was 11°C for the gold coated particles and 16 °C for the acrylic acid-coated particles. For the sample with 1% particles, the temperature of both acrylic acid- and gold-coated increased approximately 4~5 times higher than those of the 0.1%. In summary, both acrylic acid-coated and gold-coated Fe₃O₄ were heated well at 0.45 MHz. Also, the material used for the surface coating does not seem to affect the heating.

Table 2

Sample	Concentration (%)	Δ Temp. (°C)
Acrylic acid-coated Fe ₃ O ₄ in agar gel	0.1	15.9
	1.0	64.1
Gold-coated Fe ₃ O ₄ in agar gel	0.1	10.7
	1.0	60.5

To emulate the situation of a tumor containing nanoparticles, acrylic acid- or gold-coated Fe₃O₄ were evenly mixed with 4 g of ground beef at a concentration of 0.1 and 1% and the AC EM field was applied to the sample (Table 3). At a concentration of 0.1%, the temperature of particle containing beef increased by approximately 16°C, while at 1%, by 60°C. In all cases, the temperature increase of the meat without particles was minimal.

Table 3

Sample	Concentration (%)	Δ Temp. (°C)
Acrylic acid-coated Fe ₃ O ₄ in ground beef	0.1	16.0
	1.0	64.8
Gold-coated Fe ₃ O ₄ In ground beef	0.1	10.4
	1.0	60.2

AEM field distribution and applicator configuration were studied. Theoretically, if these particles are accumulated at the tumor site, only tumor can be heated effectively without heating surrounding healthy tissue. Therefore, the heating of accumulated particles in breast tissue model was studied. Cylindrical breast models 40 were constructed with agar gel at a dimension of 10 (diameter) x 7 cm (height). Vitamin E capsule shells (2.5 x 1.0 x 1.0 cm) filled with 1% of Fe_3O_4 in agar gel was placed at 1 and 2.5 cm deep in the agar breast model 40. A ring shape (inner diameter of 11 cm with 3 turns) or pancake (3 or 10 cm diameter with 4 turns) shaped induction heater coils 42 were applied around/on the tumor model 44 containing breast models 40 (FIGs. 10a and 10b). An AEM generator 46 is also provided.

Table 4 shows the effect of the induction coil geometry on the tumor model containing iron oxide nanoparticle in tissue model. With the ring shaped induction coil at a diameter of 10 cm, after 10 minutes of heating, very little heating was observed for the tumor, 1 or 2.5 cm deep. With the pancake shaped coil at a diameter of 10 cm, little temperature increase was observed after 10 minutes for both the tumor at 1 and 2.5 cm deep. For the pancake shaped induction coil at a diameter of 3 cm, however, the tumor temperature at 1 cm deep increased by 17°C only after 2 minutes and even at 2.5 cm deep, the tumor temperature increased by 1°C. In all cases, no temperature increase was observed for the control. Therefore, the geometry of the induction coil, in other words, the AEM field strength, is a very important factor to be considered to optimize the heating of nanoparticles.

Table 4

Induction coils	Diameter (cm)	Heating time (Min.)	ΔT (°C)	
			1 cm deep	2.5 deep
Ring Shape	10	10	2	0
Pan Cake Shape	10	10	0	0
	3	2	17	1

AEM field distribution vs. applicator configuration were studied. To study the AEM field penetration depth in breast tissue, computer simulations of AEM field were performed using a computer code. The respective fields 48a, 48b around a single and a dual pancake type coil(s) were simulated (FIGs. 11a and 11b). The level

of brightness shows the density of magnetic flux. For the single pancake shaped coil, the flux density seems to be distributed along the coils, with little diffusing to the neighboring areas (FIG. 11a). For the two pancakes system, with the same current flow directions for both, the magnetic flux density between the coils are enhanced greatly (FIG. 11b).

Heat conduction from heated tumor to normal tissue was studied. To understand the heat conduction from nanoparticle containing tumors to the normal tissue, the following experiment was conducted. To form a particle containing tumor model, ground chicken meat was mixed with Fe_3O_4 particles at 10~20 nm at weight % (cylindrical shape; 1.2 x 2 cm). The tumor model was placed either under the AEM coil in the air or in chicken breast tissue. The distances between the AEM applicator and the center of the tumor model were adjusted as 1.3, 1.8, and 2.3 cm (0.3 cm was for the heat insulator). Then AEM field at a frequency of 400 KHz, and power at 3 KW, was applied to the system by a pancake shaped coil (3 turns x 3 cm I.D.), for 10 minutes.

For the tumor model in the air, the temperature increases were 6.8, 2, and 0.9°C for the distance from the coil and the tumor model at 1, 1.5, and 2.0, respectively (FIG. 12). For the tumor model in the meat (chicken breast tissue), the temperature increases were 4.2, 1, and 0°C for the tumor depth at 1, 1.5, and 2.0, respectively, approximately 40% less than those of tumor model in the air. For both cases, the magnetic field intensity seems to decrease exponentially from the induction coil. Large amount of heat generated by the nanoparticles in the tumor model was absorbed by the surrounding meat, by conduction. This result illustrates that, to effectively perform nanoparticle guided hyperthermia with minimal heat conduction to the normal tissue, the magnetic field strength needs to be well adjusted depending upon the depth of the tumor. This result also illustrates the need for mathematical modeling, for better predicting the magnetic field strength, and appropriate nanoparticle heating rate as well as heat dissipation rate to the normal tissue.

The affinity of LHRH to a receptor with and without linking to NGPs was studied. For the cancer treatment, various moieties have been examined, including sugars, lectins, receptor ligands, and antibodies. LHRH receptor was

cloned, also known as gonadotropin-releasing hormone (GnRH) receptor from human pituitary and breast tumors. Researchers including our group have found that LHRH receptors are overexpressed in breast, ovarian, endometrial, and prostate cancer cells. In contrast, LHRH receptors are not expressed, or expressed at a low level in most visceral organs. Binding of LHRH to its receptors increases: (i) intracellular cAMP, (ii) phosphoinositol triphosphate (IP3), (iii) MAPK activity, and (iv) it causes internalization of LHRH-receptor complex.

LHRH has an amino acid sequence of pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH₂. It can be therefore bound to the surface of nanogold particles spontaneously by its N-terminal amine group *via* self-assembled process. [D-Trp⁶]LHRH is a decapeptide analog of LHRH that is currently used for the treatment of sex-hormone-dependent tumors including breast cancer. [D-Trp⁶]LHRH (MW = 1311.45), was coated on the surface of 10 nm sized nanogold colloids. After the reaction, a dialysis tube was used to remove unreacted [D-Trp⁶]LHRH. The [D-Trp⁶]LHRH coated nanogold particles (Trp-NGP) was adjusted to pH 9.0 using 0.1 mM sodium carbonate solution.

To test the binding of [D-Trp⁶]LHRH to gold coated particles and retention of their binding ability to [D-Trp⁶]-LHRH receptors and its biological function, we examined the binding affinity of LHRH-NGPs for their binding to high affinity LHRH receptors. For this purpose, we used mouse gonadotrope cell line (LβT2) that express high levels of high affinity LHRH receptors. The LβT2 cells were plated on 6-well plates. After 24 hour of plating, cells were transfected with reporter gene construct CRE-Luciferase (1 μg/well) as described previously. After 24 hours of transfection, the medium was replaced with serum free medium and incubated for 60 minutes followed by treatment of cells with various concentrations of [D-Trp⁶]LHRH or trp-NGP for four hours. Cells were lysed and assayed for luciferase activity. Binding of [D-Trp⁶]LHRH or Trp-NGPs was calculated. Experiments were repeated twice.

Binding affinity (Iz₅₀) value for [D-Trp⁶]LHRH-linked to gold particles was found to be similar to native [D-Trp⁶]LHRH peptide (0.1 nM), suggesting that binding of LHRH analog to gold particles retain its binding to receptor and is

biological activity and did not change its binding and affinity for the receptor compared to native analog. These results suggest that [D-Trp⁶]LHRH-gold particles can be very useful tool for the treatment and diagnosis of breast cancer.

Preliminary study results indicate that the magnetic core appears to be the main cause of heating by the AEM wave. With respect to nanoparticle core size, particles at the size range 10~30 nm appearing to provide the best heating effect.

With respect gold layer thickness, the plasmon resonance of metal nanoshells can vary by changing the shell thickness of gold and the size of the dielectric core. Thick shells around small cores give rise to resonance in the visible region, while the shallow shells around large cores shift the resonance frequency to the near infrared region. It has been that observed a red shift of the plasmon resonance frequency of gold-coated iron nanoparticles. The gold particles at around 150 nm showed very good absorption at 780nm. Therefore, the absorption of the gold-coated magnetic nanoparticles may be tuned to the near infrared region, by adjusting their core size and shell thickness. The thickness of the gold shell may also affect the heating capacity of the magnetic nanoparticles. The heating effect with respect to the gold shell thicknesses and also the ratio between the core and the gold shell thickness will be studied. The SAR values will be measured for both magnetic nanoparticles with/without gold coating. Optical contrast by these particles will also be measured in NIR -TRS.

As shown in the preliminary study results, NGP can enhance fluorescence extensively. The fluorescence enhancement of the safe fluorophore ICG (cypate), on the gold surface will be tested for both its absorption and fluorescence. Since a different sized nanogold particle (NGP) has different plasmon field intensity, various sizes of NGP (2~50 nm) and various spacer thicknesses (1~5 nm) will be tested to select the best combination of the NGP size and spacer length.

Electroless coating (chemical method) can be an effective approach for coating of metals such as gold on the nanoparticle. As can be seen in preliminary study results, this method may be used for gold coating on the surface of Fe₃O₄ particles. Electroless plating using Co (II) as a reducing agent is a novel method

developed in recent years. The complete gold thin film can be easily formed by this solution coating method.

Gold layer thickness is varied by changing the relative concentration of nanoparticles to metal salt solutions.

5 Plasma coating of biocompatible, hydrophilic polymer on the gold layer proceeded as follows. The monomers with which the inventors have extensive experience include pyrrole, acrylic acid, and acetylene. By varying their deposition conditions between high power/low pressures to low pressure / high power, the properties are varied from high modulus/high crosslink density to low modulus/low
10 crosslink density. These monomers have been used extensively previously and therefore, their deposition characteristics and structure-property relationships for DC and RF films are known.

Plasma polymerization depends on the following parameters: (1) monomer flow rate; (2) system pressure; (3) discharge power among other variable
15 parameters such as the geometry of the system; (4) reactivity of the starting monomer; (5) frequency of the excitation signal; and (6) temperature of the substrate. The processing parameters may be varied in order to achieve the ideal coating thickness, surface morphology, and structures of the polymer films. By correlating the film properties and plasma conditions, the processing parameters will be optimized.

20

Development of the alloy particle proceeded as follows.

In a first method, alloys of iron/nickel and nickel/copper tend to have Curie temperatures in our range of interest. $\text{Ni}_{71}\text{Cu}_{29}$ alloy has been used previously for hyperthermia applications. This method, however, does not lend itself to
25 additional particle modification processes easily. Given the excellent solubility of iron/nickel and nickel/copper alloys, these systems is ideal for solution phase synthesis of core/shell nanoparticles. The micro emulsion method uses a surfactant to form a micelle in solution. The micelle is then used as the reaction chamber for nanoparticle synthesis and plays a role in determining the ultimate size of the particle.
30 In this case, a surfactant will be utilized, such as CTAB, and a co-surfactant, such as n-hexanol or n-butanol, to form the micelles. Metals are added to the micelle in the

form of a metallic salt, either metal chloride or metal sulfate. To reduce metal ions to a metallic alloy, a reduction agent such as sodium borohydride is added to the solution.

In an effort to gain a greater degree of control over the reaction, independent solutions of the metal salt and the reduction agent are created. The two solutions are then reacted in another reaction vessel, and are allowed to react under an argon atmosphere. Excess reduction agent is to be added to system to help avoid oxidation of the alloy by the water in the micelle core. The water to surfactant molar ratio (ω) is used to control the size of the micelle core and, hence, the size of the nanoparticle. The benefit to this system is that by simply adding an aqueous solution of the gold salt, in this case hydrogen tetrachloroaurate, and some additional reducing agent, the micelles will expand and allow for the synthesis of the gold shell while still in solution under the argon atmosphere. This will help to further limit potential oxidation of the magnetic alloys. This procedure has been utilized for a number of particle systems, including CoPt_x/Au, Fe/Au and Co/Au.

Metals of interest are iron/nickel and nickel/copper alloys. To synthesize these nanoparticle cores, an aqueous solution of the metal ions will be created. The molar ratio of the metal required in the alloy will be mirrored in the molar ratio of the ions in solution. In the case of Ni₇₁Cu₂₉, the molar ratio will be 79:21 nickel to copper ions. A second aqueous solution will be prepared using an excess of the reducing agent NaBH₄. The size of the micelle is set by ω , which will play a role in determining the size of the nanoparticle. Two feed solutions are created using an identical ratio of solvent (octane), surfactant (CTAB), co-surfactant (1-Butanol) and the aqueous solutions to insure a consistent particle size. These solutions are then combined under an argon atmosphere and allowed to react. The solution should change color, which would indicate formation of the nanoparticle species.

In a second method, Ni (71%)/Cu (29%) alloy can also be made by physical melting. Nickel and Copper powders are mixed with ball milling and the smaller powder and higher energy ball milling, the better the results. Then compacting and heating them at 1460 °C under inert gas condition. The phase

equilibrium system for copper-nickel shows a linear progression for the Curie temperature, which starts at a composition of 67% nickel and 33% copper (by weight) for a temperature of 0°C. From the phase diagram of Cu-Ni alloy, the optimum amount of nickel in the alloy is determined to be 71–71.4% by weight to have a Curie temperature in the desired range of 41–46°C. By controlling the composition and synthesis temperature, we will be able to obtain particles with deferent sizes down to 20 nm. Processing parameters will be optimized in terms of particle size, composition and surface morphology.

In a third method, control of the composition in the nano-level is difficult, since molecules and atoms in common techniques (e.g. chemical vapor deposition, plasma vapor deposition) do not necessarily arrange in the preferred composition, which is determined on bulk material on the macroscopic level. In this method, a simple process is used that combines melting and ball milling of bulk materials. Ball milling (mechanical alloying) was reviewed, and its impact on nanostructured materials, indicating that ball milling can produce average grain sizes below 100 nm.

Solid state synthesis of these powders can be performed by ball-milling of MnO_2 , La_2O_3 and SrO , taken in the stoichiometric ratio, with ZrO_2 balls in ethanol for 24 h followed by annealing for 40 h in air at 1000°C and for 20 hr at 1150°C in air with intermediate grinding. In order to avoid the appearance of secondary phases due to crystallization during sintering, additional heat treatment at 1300°C for 10 hr needs to be applied to samples.

A stock solution for freeze-drying (FD) synthesis will prepared by mixing of appropriate amounts of pre-analyzed 0.3-0.5 M solutions of La and Mn acetates and Sr nitrate. The solution will sprayed by pneumatic nozzle (mean diameter of droplet=100-200 mm) into liquid nitrogen under intensive stirring, then tray-dried in a semi-industrial freeze-drier SMH-15 at $P=4 \times 10^{-2}$ mBar for 48 hr; the temperature of the heating plates will be changed from -50 to +40°C. Thermal decomposition of salt powders, obtained by freeze-drying, will be performed by slow heating (1 K/min) of the powder in air up to 650°C with a further 5 hr annealing.

In a fourth method, the sol-gel methods have many advantages due to the lower synthesis temperatures and the finer and more homogeneous particles produced. However, to achieve the compositional homogeneity of the final oxide powder, the preparation of a homogeneous gel with respect to the distribution of cations is very important. Therefore, a suitable precursor solution must be prepared which can be converted to a gel without any cation segregation.

In the method, the sol-gel technique for the synthesis of $Y_3Fe_{5-x}Al_xO_{12}$ will be used, through two different complexing agents (citric acid and malonic acid) and adding two different alcohols (ethylene glycol and glycerol). A solution 0.2 M $Fe(NO_3)_3 \cdot 9H_2O$, 0.12 M $Y(NO_3)_3 \cdot 5H_2O$, and x M (x = 1, 2, 3 M) citric acid, was heated at 80 °C in order to obtain the gel. The citric acid is added to the gel to adjust pH = 2. This gel will be dried at 110 °C for 36 h and further heat treated in air at temperatures between 400 and 1000°C and for periods of time between 2 and 24 hr, with a heating rate of 10°C/min. This sol-gel method leads to $Y_3Fe_{5-x}Al_xO_{12}$ nanoparticles which crystallize at lower temperatures (650°C) and the intermediate compound $YFeO_3$ does not appear. The proposed study will carry out on $Y_3Fe_{5-x}Al_xO_{12}$ nanoparticles primarily to tailor its Curie temperature (T_c) at around 50°C by substitution of iron by diamagnetic aluminum in $Y_3Fe_{5-x}Al_xO_{12}$.

Mathematical modeling and computer simulation for AEM field strength and for the temperature distribution in tissue was used. Magnetic field strength will be mathematically computed with respect to the distance from the AEM applicator, to predict the optimal field strength for hyperthermia. The heat transfer in a breast tissue with a breast at various particle concentrations and depths of the tumor location will be mathematically simulated and also experimentally verified.

Magnetic strength, H (in units of A/m) can be mathematically expressed as,

$$\nabla \times \mathbf{H} = \mathbf{J} + \partial \mathbf{E} / \partial t \quad (1)$$

where E and t are electric field strength and the time, respectively. H is related to the magnetic flux (B) divided by the permeability (μ). J, the current density, is defined by $\mathbf{J} = \int \rho_q \mathbf{v} dV$, where $\mathbf{v}$ is a vector, the drift velocity of the charge carriers that have a density described by the scalar function ρ_q . V is the volume of interest.

Considering the average breast thickness between two mammogram plates is 5 cm, the deepest AEM penetration position is 2.5 cm. Parameters to study for the proper AEM field strength are:

- 5 (1) Size of the induction heater: For the given frequency and input power, the magnetic field intensity will depend on the size of the coil. 3~10 cm sizes of pancake shaped induction coil are considered
- (2) Number of turns: various numbers of coil turns are considered.
- (3) Thickness of coil: various thickness of coil (diameter of coil: 0.25, 0.5, and 0.75
10 cm) are considered.
- (4) Frequency: For the electromagnetic frequency, the rule of thumb is that the lower the frequency, the deeper the field penetration. Therefore, the frequency effect on its penetration to the breast model (reflectance) was studied in the frequency range between 50 and 500 KHz. .
- 15 (5) Input power: the magnetic field intensity generated by induction heaters will be proportional to the input power. The power level will be determined in such a way that a sufficient level of EM energy can penetrate at least 2.5 cm of the depth for the breast.
- (6) Time interval of input power on-off: The time interval of applying EM field will
20 be estimated to keep the tumor temperature constant.

When the AC magnetic field is applied to the breast, magnetic nanoparticles in the tumor generate heat. Therefore, the tumor will be the main source of heat in a breast, and the heating capabilities of tumor will be calculated by tissue
25 SAR. When the samples are placed in the AC magnetic field, the heating capabilities of the samples are often expressed as SAR.

$$SAR = C \bullet \Delta T / \Delta t \quad (2)$$

where C is the specific heat capacity of the sample, T is the temperature, t is time, and
30 $\Delta T / \Delta t$ is the initial slope of the temperature versus time dependence. When magnetic

nanoparticles are dispersed in a gel or a liquid, the above equation can be expressed as follows.

$$SAR = (C \bullet m_s \bullet \Delta T / \Delta t) / m_i \quad (3)$$

5

where m_s is the mass of the sample, m_i is the total magnetic nanoparticle mass in the suspension media.

Therefore, the absorbed power for the tumor can be express as follows.

10

$$P = SAR m_i / V \quad (4)$$

where V is the volume of the tumor.

SAR values of magnetic nanoparticles depend on various factors such as size, size distribution, shape and chemical composition of the particles, frequency and amplitude of the applied magnetic field *etc.*

As can be seen in the study with heat conduction in FIG. 12, the heat conduction from the heated tumor to the normal tissue can be significant. One effective way of heating the tumor with minimal heat conduction to the normal tissue may be heating the particles very fast and stop applying the AEM field for a while so that the normal tissue gets cooled down by blood flow and then apply the field again. The appropriate heating rate depends on the particle concentration in the tumor, the distance between the tumor and the AEM applicator (or field strength), and the time of the field application, assuming all other heat transfer parameters in the breast tissue system are relatively constant, within a short time.

The temperature in the breast tumor containing nanoparticles may be first theoretically predicted by mathematical modeling and computer simulation. Mathematical models for the temperature distribution in the breast will be developed, and the effect of the tumor depth and concentration of the magnetic particles in the tumor will be evaluated with the field strength given by Eqs. 2~5. For the heat transfer, the governing equation will be used as follows.

$$\frac{\partial T}{\partial t} = \alpha \left(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2} \right) + P \quad (5)$$

Where α is the thermal diffusivity and P is the power absorbed by the nanoparticles in the tumor from the AC magnetic field. The initial temperature will be the body temperature, 36°C. The boundary temperature of the system will be taken as SAR_m /Cm_s.

Using these models, the minimal concentration of nanoparticles needed for effective hyperthermia will be estimated for a tumor at a certain depth at a specific applicator.

While various embodiments of the present invention have been shown and described, it should be understood that other modifications, substitutions and alternatives are apparent to one of ordinary skill in the art. Such modifications, substitutions and alternatives can be made without departing from the spirit and scope of the invention, which should be determined from the appended claims.

Various features of the invention are set forth in the following claims.

CLAIMS:

1. A particle for introduction into a biological or physiological system for enhancing optical contrast between target cells and surrounding tissue, and for inducing cell death in the target cells while minimizing effects on surrounding healthy tissue, said particle comprising:

a magnetic core;

a metal nanolayer surrounding said magnetic core;

a surfactant layer surrounding said metal nanolayer;

at least one specificity biomolecule having an affinity and specificity for the target cells, said specificity biomolecule bound to said metal nanolayer; and

a therapeutic agent bound to said metal nanolayer to induce cell death.

2. The particle of claim 1, said magnetic core comprising nanoparticles of one of the group consisting of Fe_2O_3 , Fe_3O_4 , alloys of iron/nickel and alloys of nickel/copper.

3. The particle of claim 1 wherein a property of said magnetic core has a Curie temperature of between approximately 40 °C and 50 °C.

4. The particle of claim 1 wherein said magnetic core is sized and configured to produce thermal energy when exposed to an alternating electromagnetic field.

5. The particle of claim 1, said metal nanolayer comprising one of the group consisting of gold, copper, silver and platinum.

6. The particle of claim 1, said surfactant layer including one of acrylic acid, acetylene, pyrrole.

7. The particle of claim 1, said at least one specificity biomolecule comprising LHRH, monoclonal antibodies against tumor markers, and aptamers.

8. The particle of claim 1 wherein said therapeutic agent comprises one of the group consisting of doxorubicin, taxol and tamoxifen.

5 9. The particle of claim 1 further comprising a fluorescence enhancer bound to said metal nanolayer.

10. The particle of claim 9 wherein said fluorescence enhancer comprises a fluorophore selected from the group consisting of idocyanine green, cypate, rhodamine and fluorescein.

11. A method of detecting and treating cancer comprising:
introducing said particle of claim 1 into a body;
exposing said particle to an electromagnetic field to induce hyperthermia in the
15 target cells.

12. A non-invasive method of simultaneously locating and destroying target cells comprising:

forming a cytotoxic particle by
20 providing a magnetic nanoparticle;
surrounding the magnetic nanoparticle with a metal nanolayer having high surface plasmon density;
surrounding the metal nanolayer with a surfactant layer;
binding at least one therapeutic agent and at least one specificity
25 biomolecule to the metal nanolayer;
introducing the cytotoxic particle to a biological or physiological system;
observing optical contrast to determine a location of the target cells; and
exposing the target cells to an alternating electromagnetic field to induce
hyperthermia in the target cells.

13. The method of claim 12 further comprising providing a magnetic nanoparticle having a Curie temperature between approximately 40°C and 50 °C.

14. The method of claim 12 further comprising selecting the metal
5 nanolayer to be from the group consisting of gold, copper, silver and platinum.

15. The method of claim 12 further comprising selecting the surfactant layer to be from the group consisting of acrylic acid, acetylene and pyrrole.

10 16. The method of claim 12 further comprising selecting the specificity biomolecule to be from the group consisting of LHRH, monoclonal antibodies against tumor markers and aptamers.

17. The method of claim 12 further comprising selecting the therapeutic
15 agent to be one of the group consisting of doxorubicin, taxol and tomosifen.

18. The method of claim 12 further comprising binding a fluorophore to the metal nanolayer.

20 19. The method of claim 12 wherein introduction of the cytotoxic particle includes injection via syringe, intratumoral introduction, intravenous introduction, suppository introduction and transdermal introduction.

25 20. The method of claim 12 wherein the target cells are exposed to an alternating electromagnetic field of equal to or less than 1 MHz.

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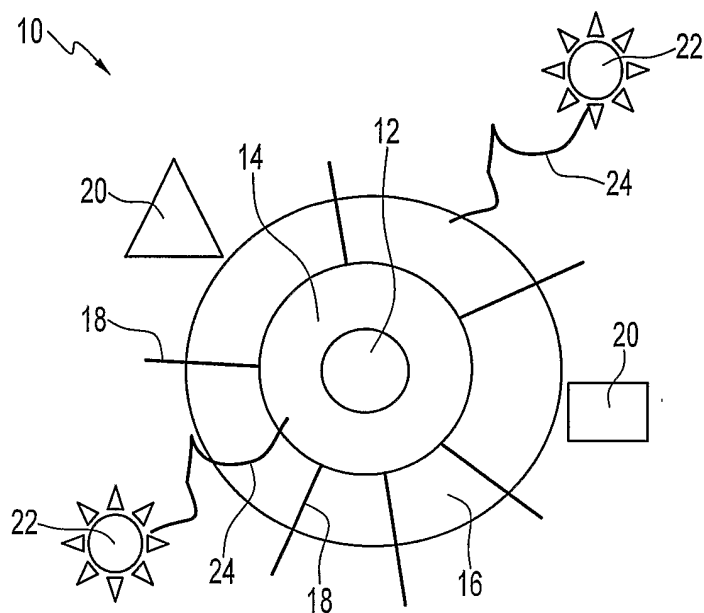


FIG. 1

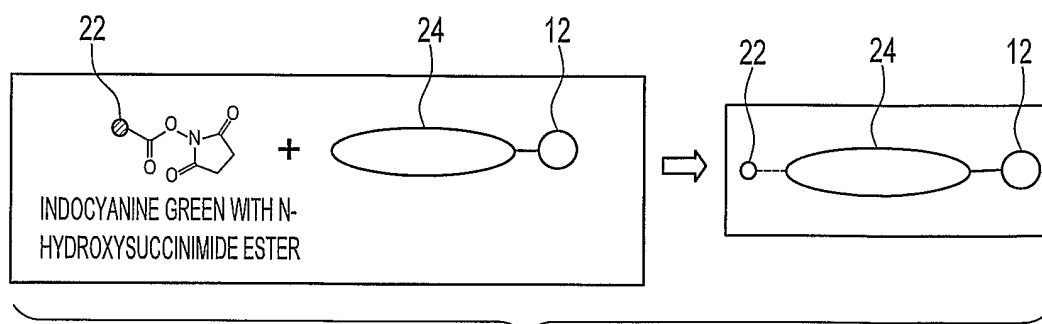


FIG. 2

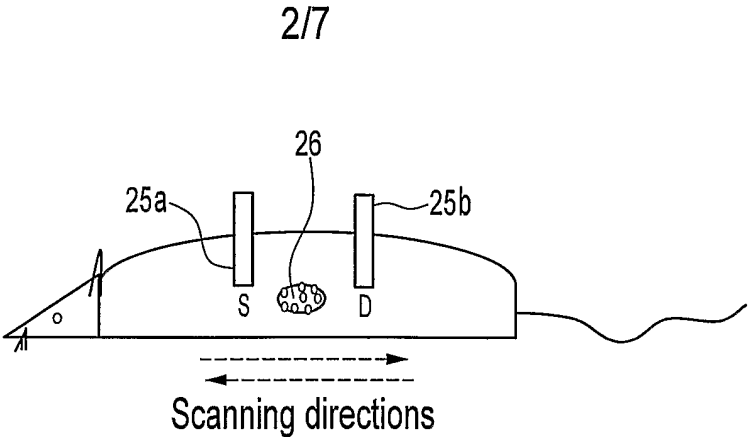


FIG. 3A

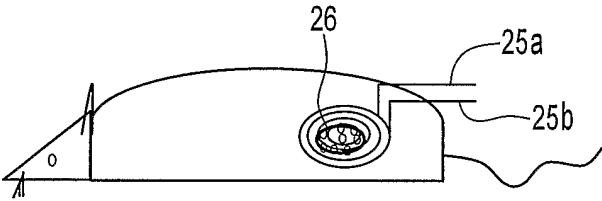


FIG. 3B

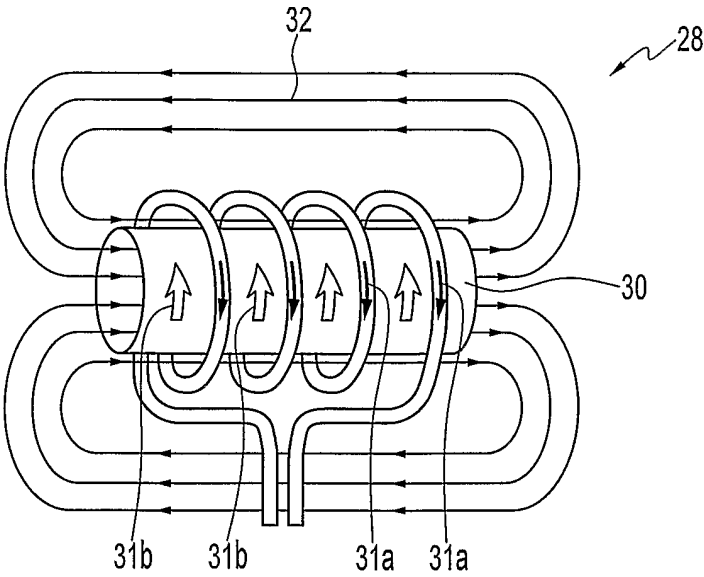


FIG. 4

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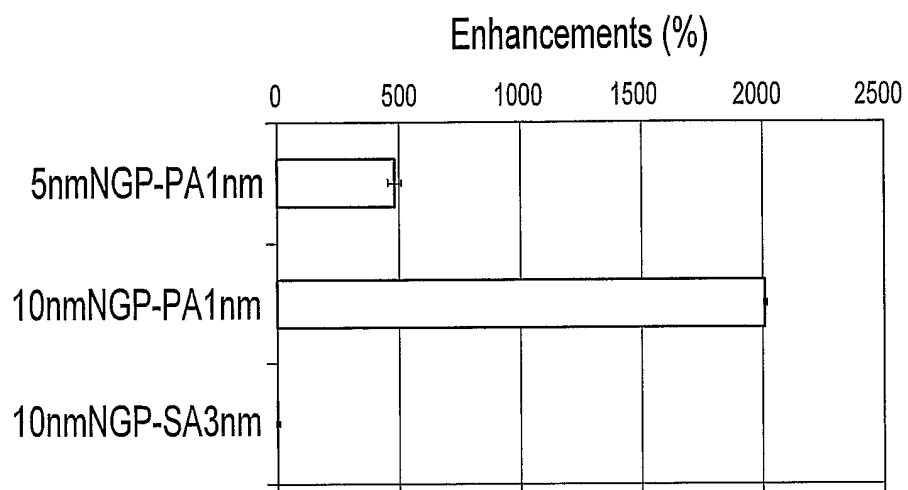


FIG. 7A

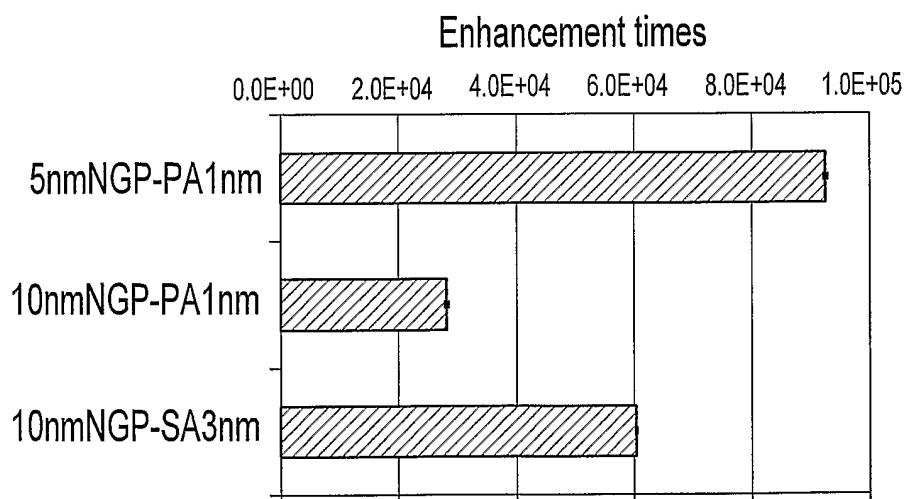


FIG. 7B

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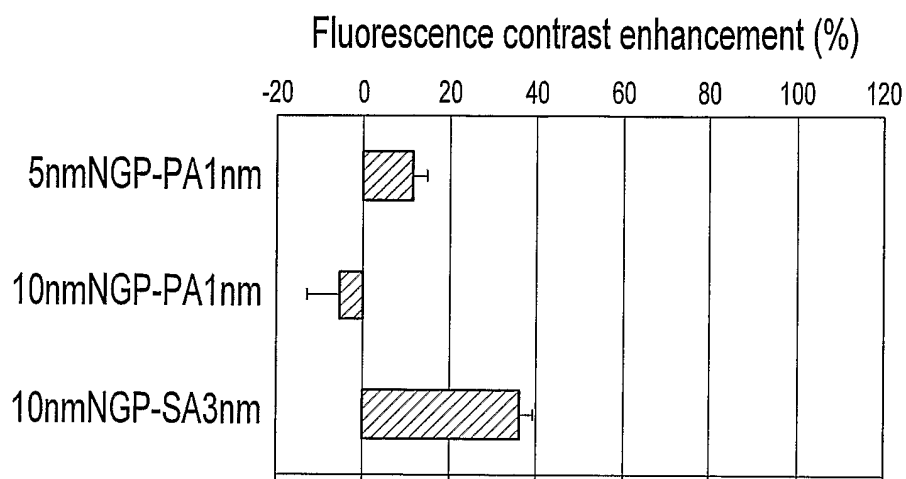


FIG. 8A

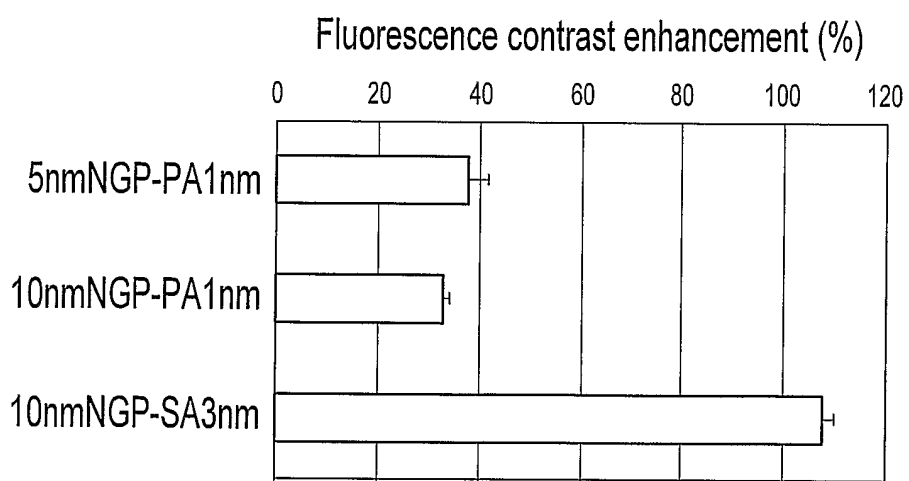


FIG. 8B

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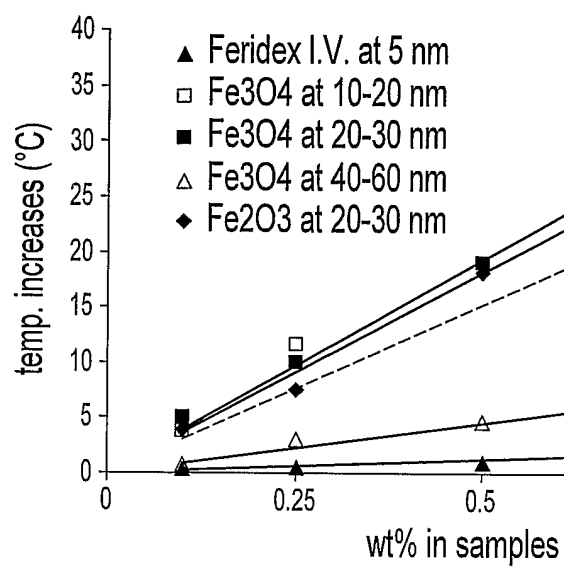


FIG. 9

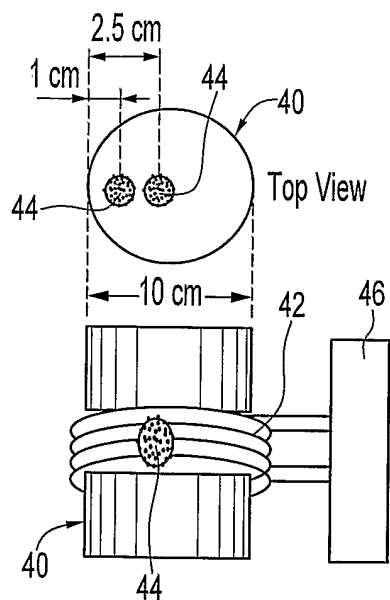


FIG. 10A

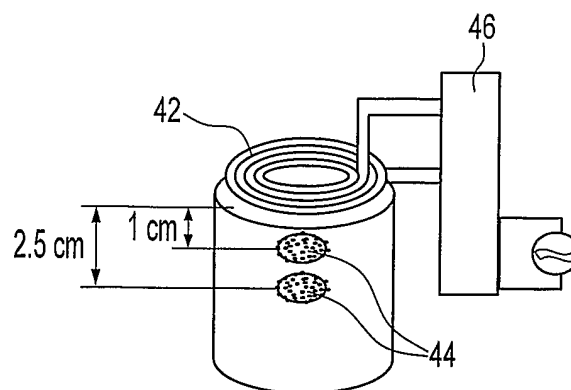


FIG. 10B

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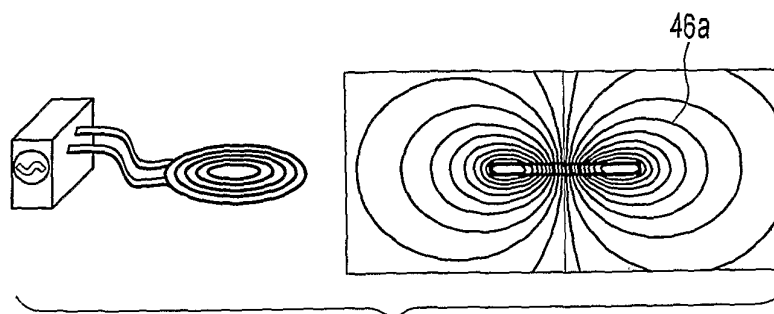


FIG. 11A

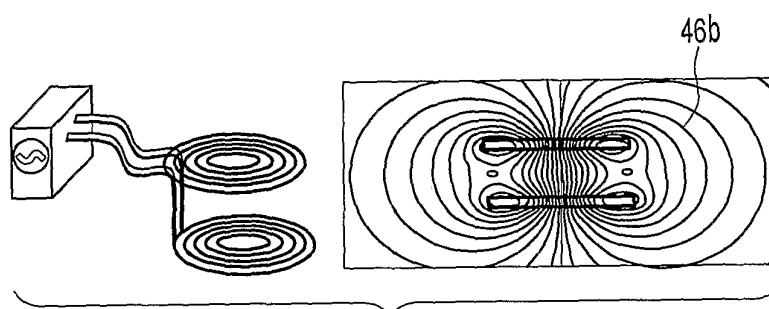


FIG. 11B

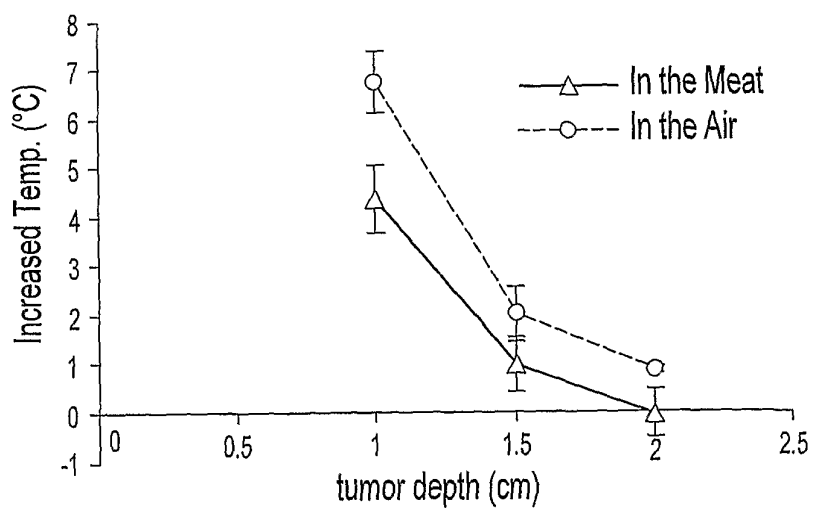


FIG. 12