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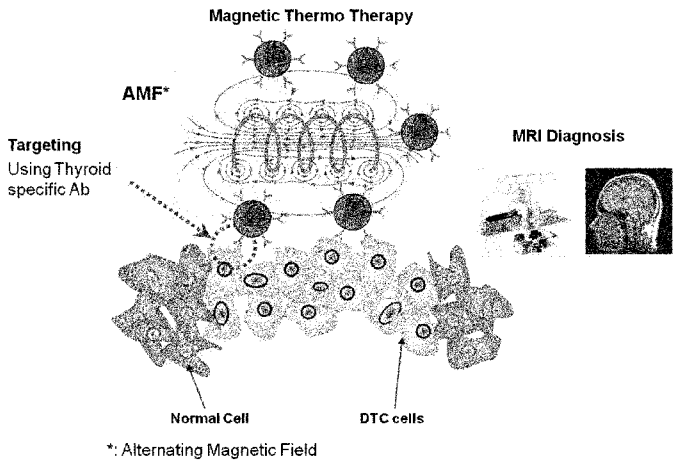
(54) **Title**: MAGNETIC NANOCOMPOSITE SPECIFIC FOR THYROID CANCER AND USE THEREOF

[Fig. 2]



(57) **Abstract**: The present invention relates to a thyroid cancer-specific magnetic nanocomposite, and more particularly, to a magnetic nanocomposite, in which magnetic nanoparticles are encapsulated in a biocompatible polymer and a thyroid cancer-specific polypeptide is conjugated to the biocompatible polymer, a diagnostic composition for thyroid cancer comprising the magnetic nanocomposite, a therapeutic composition for thyroid cancer comprising the magnetic nanocomposite, a method for providing information for thyroid cancer diagnosis using the magnetic nanocomposite, and a therapeutic method thereof. The magnetic nanocomposite of the present invention is advantageous in that it can be used for diagnosis and treatment of thyroid cancer at the same time.

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Description

Title of Invention: MAGNETIC NANOCOMPOSITE SPECIFIC FOR THYROID CANCER AND USE THEREOF

Technical Field

[1] The present invention relates to a thyroid cancer-specific magnetic nanocomposite, and more particularly, to a magnetic nanocomposite, in which magnetic nanoparticles are encapsulated in a biocompatible polymer and a thyroid cancer-specific polypeptide is conjugated to the biocompatible polymer, a diagnostic composition for thyroid cancer comprising the magnetic nanocomposite, a therapeutic composition for thyroid cancer comprising the magnetic nanocomposite, a method for providing information for thyroid cancer diagnosis using the magnetic nanocomposite, and a therapeutic method thereof. The magnetic nanocomposite of the present invention is advantageous in that it can be used for diagnosis and treatment of thyroid cancer at the same time.

Background Art

[2] Thyroid cancer has been the most common female cancer in Korea since 2005, and there are over 20,000 new patients annually. About 80-90% of thyroid malignancies are papillary thyroid cancer which has well-differentiated carcinomas and has an excellent prognosis. The standard therapeutic strategy is as follows: 1) partial thyroidectomy when papillary thyroid cancer diagnosed and total thyroidectomy for thyroid cancer greater than or equal to 1 cm; 2) remnant ablation with radioactive iodine for thyroidectomy patients; 3) high dose I-131 therapy for distant metastasis (lung or bone); and 4) for recurrent cases, surgical excision, followed by high dose I-131 therapy.

[3] The theoretical background for the therapeutic strategy is that about 75% of differentiated thyroid cancer expresses a cell surface protein, called sodium-iodine symporter (NIS), which is responsible for iodine uptake into the cytoplasm, and thus high levels of ingested radioactive iodine can be accumulated in cancer cells. It can be an ideal treatment strategy as a diagnostic and therapeutic tool because one can evaluate the effectiveness of the therapy as well as the extent of the disease or distant metastasis by a whole-body scan after intake of radioactive iodine. However, radionuclide has a limited dosage, and is accumulated in the salivary gland to cause fibrosis, resulting in salivary gland hypofunction. Immunosuppression can also occur even though it is rare. Additionally, patients have to stop taking the thyroid hormone medication for about 2 weeks to maximize the therapeutic effect as well as to eat a low-iodine diet to ensure optimal iodine accumulation. All of these preparations cause significant pain and discomfort to the patients.

[4] Thermotherapy using magnetic nanoparticles (magnetite) has produced successful therapeutic results for brain tumors, prostate cancer and skin cancer according to data from animal studies. Magnetite nanoparticles have the property of producing heat by oscillating under an alternating magnetic field (AMF). Using this property, thermotherapy is performed under an alternating magnetic field through direct injection into tumor tissues or distribution of a high level of a specific antibody-conjugated form in tumor tissues. It is crucial that cancer tissue express the specific antigen homogenously and exclusively for effective thermotherapy using antibody-conjugated magnetite nanoparticles. Thus, it is important to find cancer showing homogenous expression of specific antigens distinct from normal tissue for the improvement of thermotherapy using antibody-conjugated magnetite nanoparticles.

[5] Thermotherapy using magnetic nanoparticles is to induce cell death by making small holes on the surface of cancer cells, and it is a different concept from the conventional ablation therapy using high frequency, ultrasound, and laser. In addition, since magnetite nanoparticles can be enhanced in T2-weighted magnetic resonance imaging (MRI), conjugation of antibody with magnetite nanoparticle allows selective accumulation in cancer cells, and thus can be used as a diagnostic tool. Until now, there have been many reports on cancer therapy using magnetic nanoparticles for various cancers, but no reports on the use of magnetic nanoparticles conjugated with thyroid cancer-specific antibody for the diagnosis and treatment of thyroid cancer.

Disclosure of Invention

Technical Problem

[6] In order to solve the problems of the conventional radioactive iodine therapy, the present inventors have made many efforts to develop effective diagnostic and therapeutic methods for thyroid cancer. As a result, they found that antibody-conjugated magnetite nanoparticles are used to diagnose and treat thyroid cancer at the same time, thereby completing the present invention.

Solution to Problem

[7] An object of the present invention is to provide a magnetic nanocomposite, comprising a magnetic nanoparticle; a biocompatible polymer encapsulating the magnetic nanoparticle; and a thyroid cancer-specific polypeptide that is conjugated to the biocompatible polymer.

[8] Another object of the present invention is to provide a diagnostic composition for thyroid cancer comprising the magnetic nanocomposite.

[9] Still another object of the present invention is to provide a therapeutic composition for thyroid cancer comprising the magnetic nanocomposite.

[10] Still another object of the present invention is to provide a method for providing in-

formation for thyroid cancer diagnosis using the magnetic nanocomposite.

[11] Still another object of the present invention is to provide a therapeutic method for thyroid cancer using the magnetic nanocomposite.

Advantageous Effects of Invention

[12] The magnetic nanocomposite of the present invention is conjugated with a thyroid cancer-specific antibody or ligand so as to specifically diagnose thyroid cancer and perform thermotherapy under an alternating magnetic field. Therefore, when it is used in a magnetic resonance imaging system capable of producing an alternating magnetic field adjusted for therapeutic purposes or an alternating magnetic field-generating apparatus installable on the conventional magnetic resonance imaging system, thermotherapy can be performed on the lesions immediately after diagnosis, thereby providing a new concept of simultaneous diagnosis and therapy for thyroid cancer. In addition, when there are technical difficulties in operating the alternating magnetic field-generating apparatus in the magnetic resonance imaging units, a patient can be transferred after diagnosis, and then treated with alternating magnetic fields. These effects make it possible to overcome the drawbacks and side-effects of the radioactive iodine therapy or radioactive therapy that is performed additionally for thyroidectomy patients or singly for distant metastasis, which will ease the pain of thyroid cancer patients.

[13]

Brief Description of Drawings

[14] FIG. 1 is a schematic illustration showing the magnetic nanocomposite synthesis, in which the magnetic nanoparticles of the present invention are coated with PEG to prepare magnetic nanoparticles with biocompatibity, after which they are conjugated with target moieties to prepare a magnetic nanocomposite;

[15] FIG. 2 is a schematic illustration showing simultaneous diagnostic and therapeutic applications of the magnetic nanocomposite of the present invention for thyroid cancer, in which binding of the thyroid-specific antibody-conjugated magnetic nanoparticles to thyroid cancer is detected by MRI, and then the diagnosed thyroid cancer is treated by thermotherapy using an alternating magnetic field;

[16] FIG. 3 is the result of flow cytometry showing the expression levels of the thyroid surface antigen NIS and TSHR in a rat normal thyroid cell line, FRTL5;

[17] FIG. 4 is the result of flow cytometry showing the expression levels of the thyroid surface antigen NIS and TSHR in a human immortalized thyroid cell line, Nthy;

[18] FIG. 5 is the result of flow cytometry showing the expression levels of the thyroid surface antigen NIS and TSHR in a human cervical cancer cell line, HeLa;

[19] FIG. 6 is the result of flow cytometry showing the binding capability of the magnetic

nanoparticles conjugated with the thyroid-specific antibodies, NIS and TSHR antibodies, in which *NIS-NP represents NIS antibody-conjugated magnetic nanoparticles, and **TSHR-NP represents TSHR antibody-conjugated magnetic nanoparticles;

[20] FIG. 7 shows binding of NIS-MNP to NIS-over expressing HeLa-NIS cells, in which (A) HeLa-NIS cells were stained with the indicated antibodies (AF488; green), TdTomato (red) was linked to the C-terminus of the transfected NIS protein, the nucleus was stained with DAPI (blue), and HeLa cells (right) were stained with NIS-MNP to ensure that the MNPs do not bind non-specifically to cells, (B) is an enlarged picture of HeLa-NIS stained with anti-NIS antibody, and (C) is an enlarged picture of HeLa-NIS stained with anti-NIS antibody conjugated to MNP, and scale bars indicate 10 μm;

[21] FIG. 8 is a graph showing temperature change of magnetic nanoparticles after exposure to alternating magnetic field;

[22] FIG. 9 shows In vitro hyperthermia of HeLa-NIS cells using MNPs, in which HeLa and HeLa-NIS cells were incubated with media alone or media containing 0.2 ㎎/㎖ of anti-NIS antibody-conjugated magnetite nanoparticles (NIS-MNP), and HeLa-NIS cells showed reduced viability (77%) compared to HeLa cells (96%) after exposure to AMF (* $P<0.001$);

[23] FIG. 10 is MR imaging of thyroid glands, in which A and B are pre-contrast T2- and T2* -weighted images, respectively, A-1 and B-1 are post-contrast (1 h) images after NIS-MNP intravenous injection, white arrows indicate the thyroid gland, and A-1 and B-1 showed definite post-contrast enhancement of the thyroid glands in comparison to the pre-contrast images;

[24] FIG. 11 shows AMF irradiation of HeLa-NIS tumor model, in which the copper coils generating alternating magnetic fields are shown around the mouse. One hour after injection of the MNPs, all mice were exposed to the AMF (frequency: 7 MHz) for one hour and again 24 hours after injection;

[25] FIG. 12 shows T2-weighted MRI images of a HeLa-NIS tumor, in which A is a pre-contrast image, necrosis is primarily located in the lower portion of the tumor, and A-1 shows tumor enhancement as a result of intra-tumoral NIS-MNP injection. The images were taken one hour after NIS-MNP injection, and NIS-MNP was successfully distributed to the tumor, even though leakage into the necrotic portion occurred;

[26] FIG. 13 shows tumor growth inhibition by NIS-MNPs under AMF irradiation, in which mice in the NIS-MNP injection group (cases 1 and 2) showed retarded tumor growth after AMF irradiation in comparison to the control group which received a saline injection, the y-axis shows the relative tumor volume ratio, and each dot indicates post-AMF irradiation periods; and

[27] FIG. 14 shows histochemical tumor evaluation, in which A shows residual NIS-MNPs that accumulated along the subcapsular region and the central necrotic portion of the tumor, white arrows indicate the positively stained area, A-1 and A-2 are x100 magnification views that show NIS-MNP deposits in the subcapsular region and central necrotic portion, respectively, B shows the control specimen, which was not injected with NIS-MNP, and Prussian blue staining was not observed in the tumor.

Best Mode for Carrying out the Invention

[28] In one embodiment to achieve the above objects, the present invention provides a magnetic nanocomposite, comprising a magnetic nanoparticle; a biocompatible polymer encapsulating the magnetic nanoparticle; and a thyroid cancer-specific polypeptide that is conjugated to the biocompatible polymer.

[29] A schematic illustration of the magnetic nanocomposite of the present invention is shown in FIG. 1. As shown in FIG. 1, in the magnetic nanocomposite of the present invention, magnetic nanoparticles are encapsulated by the biocompatible polymer, and the biocompatible polymer is conjugated with a thyroid cancer-specific polypeptide.

[30] As used herein, the term "magnetic nanoparticle (MNP)" means a metal nanocomposite having magnetic properties, and may be composed of a magnetic nanoparticle, metal, magnetic substance, or magnetic alloy. The magnetic nanoparticle may include any substance that can constitute magnetic nanoparticles without limitation, and examples thereof may include magnetic nanoparticles having a spinel structure or an inverse spinel structure. Specifically, examples thereof may include iron oxides (Fe_2O_3, Fe_3O_4), metal alloys (FePt, CoPt) or ferrites ($MnFe_2O_4$, $CoFe_2O_4$, $NiFe_2O_4$ or $ZnFe_2O_4$), and preferably iron oxides. Due to magnetic properties, the magnetic nanoparticles can be used as a tool for cancer diagnosis by magnetic resonance imaging (MRI) contrast effect.

[31] The magnetic nanoparticle may be preferably a uniform magnetic nanoparticle having a diameter of 1 to 200 nm, and most preferably 12 nm.

[32] As used herein, the term "biocompatible polymer" refers to a substance capable of lowering the toxicity of the magnetic nanoparticles, and means a substance that encapsulates and coats the surface of the magnetic nanoparticles to ensure the stability of the magnetic nanoparticle in vivo. The substance that binds to the surface of the magnetic nanoparticles to endow them with biocompatibility includes any substance without limitation, and it may be exemplified by polyethylene glycol (PEG), polypropylene glycol, Pluronic (polyethylene glycol-polypropylene glycol-polyethylene glycol copolymer), polyetherimide (PEI), polyvinylpyrrolidone (PVP), N-isopropylacrylamide (NIPAM), hexa-decyltrimethylammonium bromide (CTAB), or mixtures thereof, and is preferably polyethylene glycol. According to one em-

bodiment of the present invention, the magnetic nanoparticles are coated with polyethylene glycol to prepare PEGylated magnetic nanoparticles (PEGylated MNP) (see Example 2). PEGylated magnetic nanoparticles have been approved by the US FDA for their safety in the human body. Therefore, the PEGylated magnetic nanoparticles can be directly applied to the human body for diagnosis and treatment.

[33] The biocompatible polymer contains one or more functional groups, and preferably 1 to 3 functional groups capable of binding with thyroid cancer-specific polypeptides. In the absence of the functional groups in the intrinsic structure of the biocompatible polymer, necessary functional groups can be added as needed.

[34] The functional group of the biocompatible polymer, which reacts with thyroid cancer-specific polypeptide, may vary depending on the type of the thyroid cancer-specific polypeptide used, but is not limited, preferably includes one or more functional groups selected from the group consisting of -COOH, -CHO, $-NH_2$, -SH, $-CONH_2$, $-PO_3H$, $-PO_4H$, $-SO_3H$, $-SO_4H$, -OH, $-NR_4^+X^-$, -sulfonate, -nitrate, -phosphonate, -succinimidyl group, -maleimide group, and -alkyl group.

[35] The binding of the biocompatible polymer with the magnetic nanoparticle may include an ester bond, a thioester bond, an amide bond and/or a thioamide bond, and any bond may be included without limitation, as long as it provides stability.

[36] As used herein, the term "thyroid cancer-specific polypeptide" means a polypeptide capable of specifically binding to the surface protein of thyroid cancer. The polypeptide may include non-antibody polypeptides and/or antibodies, and is preferably a thyroid cancer-specific antibody.

[37] As used herein, the term "surface protein of thyroid cancer" means a surface protein that is homogenously and exclusively expressed in thyroid cancer. The surface protein of thyroid cancer includes any protein without limitation, as long as its expression is distinguished from those in other normal or cancer tissues. Preferably, it may include NIS (sodium iodine symporter) or thyroid stimulating hormone receptor (TSHR).

[38] Preferably, the non-antibody polypeptide may be a ligand of the thyroid cancer surface protein, NIS or a thyroid stimulating hormone binding to the thyroid stimulating hormone receptor.

[39] Preferably, the antibody may be an anti-NIS antibody capable of binding to NIS or an anti-TSHR antibody capable of binding to the thyroid stimulating hormone receptor. As described above, the antibody is a preferred component specific to thyroid cancer tissue in the present invention. The antibody has a property of selectively and stably binding to a specific subject, and $-NH_2$ of lysine, -SH of cysteine, and -COOH of asparaginic acid and glutamic acid in the Fc region of the antibody are usefully involved in the binding with the biocompatible polymer encapsulating the magnetic nanoparticles. The antibody may be commercially purchased or prepared by a method

known in the art. In general, immunization of mammals (e.g., mouse, rat, goat, rabbit, horse, or sheep) with a proper amount of antigen is performed once or more. After a predetermined time, when the titer reaches an appropriate level, antibodies are recovered from the serum of mammal. If desired, the recovered antibodies may be purified using the known process and stored in a frozen buffer solution until use. In addition, it is possible to prepare monoclonal antibodies from the spleen of the animal. Specific experimental conditions used in the method are well known in the art (e.g., see Nam, K.S., et al. (1990) Biochim. Biophys. Acta 1046, 89-96.). According to one embodiment of the present invention, the magnetic nanocomposite prepared by conjugation of the PEGylated magnetic nanoparticle with anti-TSHR antibody or anti-NIS antibody was found to specifically bind to a human immortalized thyroid cell line, Nthy (FIG. 6). In addition, it was also found that the magnetic nanocomposite specifically binds to HeLa-NIS, which is a cell line transfected with NIS to overexpress NIS (FIG. 7). These results support that the magnetic nanocomposite of the present invention is able to specifically bind to thyroid cancer. The use of magnetic nanocomposite capable of binding to the surface protein of thyroid cancer was first developed by the present inventors, and there were no previous reports on the application of magnetic nanoparticles in the diagnosis and treatment of thyroid cancer.

[40] As used herein, the term "thyroid cancer" is a generic name for cancer arising in the thyroid, which is a butterfly-shaped organ located in the front of the neck straddling the windpipe below the thyroid cartilage, and which produces and stores thyroid hormones and release them as needed. With respect to the objects of the present invention, thyroid cancer that can be diagnosed and treated by binding of the magnetic nanocomposite of the present invention is not particularly limited, and examples thereof may include differentiated thyroid cancer and undifferentiated thyroid cancer (anaplastic thyroid carcinoma), and is preferably differentiated thyroid cancer. Differentiated thyroid cancer is papillary thyroid cancer and expresses NIS on the surface, and thus the magnetic nanocomposite of the present invention can be used to specifically diagnose papillary thyroid cancer.

[41]

[42] In still another embodiment, the present invention provides a diagnostic or therapeutic composition for thyroid cancer comprising the magnetic nanocomposite.

[43] Descriptions of the magnetic nanocomposite and thyroid cancer are the same as above.

[44] The magnetic nanocomposite according to the present invention provides a diagnostic effect using magnetic fields and a therapeutic effect using heat at the same time, and thus is useful for the diagnosis and/or treatment of thyroid cancer. Schematic illustrations of the diagnostic method and/or therapeutic method are shown in FIG. 2.

[45] As used herein, the term "diagnosis" refers to an evaluation of the presence or properties of pathological states. With respect to the objects of the present invention, diagnosis means a diagnosis of thyroid cancer. As used herein, the term "treatment" refers to reducing or alleviating thyroid cancer or one or more symptoms caused thereby, as well as causing regression of the disease to treat thyroid cancer or preventing the progression of thyroid cancer by administration of the composition.

[46] The treatment of thyroid cancer of the present invention may be performed to destroy thyroid cancer cells by heat produced from magnetic nanocomposite by irradiation of an alternating magnetic field. That is, magnetic nanoparticles produce heat by oscillating under an alternating magnetic field to make small holes on the surface of cancer cells, leading to cell death. The therapeutic composition may be performed by thermotherapy using alternating magnetic fields produced in an alternating magnetic field-generating apparatus. The alternating magnetic field-generating apparatus may include any alternating magnetic field-generating apparatus without limitation as long as it is suitable for the purposes of the present invention. For example, a magnetic resonance imaging system capable of producing an alternating magnetic field adjusted for therapeutic purposes or an alternating magnetic field-generating apparatus installable on the conventional magnetic resonance imaging system is used to perform thermotherapy on the lesions immediately after diagnosis.

[47] According to one embodiment of the present invention, when magnetite nanoparticles were exposed to the alternating magnetic field, temperature was increased (FIG. 7), indicating the therapeutic effects of the magnetic nanocomposite of the present invention on thyroid cancer. Further, when treated with media containing NIS-MNP, HeLa-NIS cells showed reduced viability (77%) compared to the control group, HeLa cells (96%) after exposure to the alternating magnetic field (FIG. 9), indicating therapeutic effects of inhibiting cancer cell growth. Furthermore, when a HeLa-NIS tumor model was treated with the alternating magnetic field, the tumor volume was reduced compared to that of the control group (FIG. 13), and the remnant NIS-MNP was effectively removed by the intracellular RES (reticular endothelial system) (FIG. 14), indicating that it can be spontaneously removed from the body after cancer treatment.

[48] The magnetic nanocomposite according to the present invention has magnetism, and thus can be used in diagnostic methods using magnetic properties. Specifically, the diagnostic methods using magnetic properties include magnetic resonance imaging (MRI), and the magnetic nanocomposite of the present invention can be used in these diagnostic methods without limitation. The magnetic nanoparticles are enhanced on T2-weighted MR imaging, and the thyroid cancer-specific polypeptide is specifically accumulated in thyroid cancer, thereby rapidly diagnosing thyroid cancer in MR

images.

[49] Preferably, other diagnostic probes can be further included in the magnetic nanocomposite of the present invention, thereby being used as double or multiple probes. For example, if a T1 MRI diagnostic probe is coupled to the magnetic nanocomposite, T2 MRI diagnosis and T1 MRI diagnosis can be simultaneously performed. Moreover, if coupled to an optical diagnostic probe, the magnetic resonance imaging and optical imaging can be simultaneously performed, and also, if coupled to a CT diagnostic contrast agent, the magnetic resonance imaging and the CT diagnosis can be simultaneously performed. In addition, if coupled to radioactive isotopes, the magnetic resonance imaging, and the PET, SPECT diagnosis can be simultaneously performed.

[50] The T1 MRI diagnostic probe includes Gd compounds or Mn compounds, the optical diagnostic probe includes fluorescent dyes, quantum dots, or dye-labeled inorganic supports (e.g., SiO_2, Al_2O_2), the CT diagnostic probe includes I (iodine) compound, or gold nanoparticles, and the radioactive isotope includes In, Tc, or F.

[51] According to one embodiment of the present invention, obvious enhancement of the thyroid gland was observed in T2-and T2*-weighted MR images after NIS-MNP injection (FIG. 10), indicating no need of additional contrast agents in the diagnosis of thyroid cancer. Moreover, successful enhancement of the tumors was observed in the HeLa-NIS tumor model after NIS-MNP injection, indicating that it can be used as a diagnostic composition (FIG. 12).

[52]

[53] The composition of the present invention may further include a medically acceptable carrier. The carrier used in the composition according to the present invention includes carriers and vehicles typically used in the medical fields, and specifically includes ion exchangers, alumina, aluminum stearate, lecithin, serum proteins (e.g., human serum albumin), buffer substances (e.g., phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids), water, salts or electrolytes (e.g., protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts), colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene glycol and wool fat, but is not limited thereto. The composition of the present invention may further include lubricants, wetting agents, emulsifying agents, suspending agents or preservatives in addition to the above ingredients.

[54] In one preferred embodiment, the composition according to the present invention may be prepared in an aqueous solution for parenteral administration, and preferably is prepared in Hank's solution, Ringer's solution, or a physiologically suitable buffer such

as a buffered physiological saline. The aqueous suspension for injection may contain a substance for increasing the viscosity of a suspension such as sodium carboxymethyl-cellulose, sorbitol, or dextran.

[55] In one preferred embodiment, the composition according to the present invention may be in a form of a sterile injectable preparation such as an aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (for example, Tween 80) and suspending agents. The sterile injectable preparation may be also a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent (for example, a solution in 1,3-butanediol). The acceptable vehicles and solvents include mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides.

[56]

[57] The present inventors prepared a metal-oleate complex by reacting the magnetic nanoparticles with iron chlorides and sodium oleates, and prepared uniform iron oxide nanoparticles (MNP) having a size of 12 nm by treatment of oleic acid and 1-octadecene. Thereafter, the resulting magnetic nanoparticles dispersed in chloroform were mixed with polyethylene glycol to prepare PEGylated magnetic nanoparticles. Then, anti-TSHR antibody/anti-NIS antibody was conjugated thereto to prepare the magnetic nanocomposite (see Examples 1 to 3). Flow cytometry showed that the magnetic nanocomposite specifically binds to an immortalized thyroid cell line, Nthy (FIG. 6), indicating that the magnetic nanocomposite can be effectively used in the diagnosis of thyroid cancer. In addition, the temperature of the magnetic nanoparticle of the present invention increased under the alternating magnetic field (FIG. 7). More specifically, HeLa and HeLa-NIS cells were treated with media containing NIS-MNP, and exposed to the alternating magnetic field, and then their cellular growth was analyzed. As a result, the growth of HeLa-NIS cells were remarkably inhibited (FIG. 9), indicating that the target cancer can be specifically treated. Furthermore, tumors of the HeLa-NIS tumor model were enhanced by NIS-MNP treatment based on MR imaging, even though additional contrast agents were not used (FIG. 12), tumor growth was inhibited when exposed to the alternating magnetic field (FIG. 13), and the remnant NIS-MNP was effectively removed (FIG. 14), indicating that the magnetic nanocomposite of the present invention can be used to perform diagnosis and treatment at the same time.

[58]

[59] In still another embodiment, the present invention provides a method for providing

information for thyroid cancer diagnosis, comprising the steps of (a) administering the composition including the magnetic nanocomposite to a patient suspected of having thyroid cancer; and (b) detecting the presence of the magnetic nanocomposite in the patient by magnetic resonance imaging (MRI).

[60] In the method for providing information for diagnosis, signals from the magnetic nanocomposite can be detected by various kinds of equipment using magnetic fields, and preferably by a magnetic resonance imaging system (MRI).

[61] During the MRI examination, the patient is placed within a powerful magnetic field, and radiofrequency energy is applied to atomic nuclei such as protons in the body, which absorb energy and then later to emit it again after radiofrequency transmission stops. The energy is converted into signals that are computer-processed to produce images. In particular, the magnetic resonance imaging system is preferably a T2 spin-spin relaxation MRI.

[62]

[63] In still another embodiment, the present invention provides a therapeutic method for thyroid cancer, comprising the step of administering the composition including the magnetic nanocomposite to a patient suspected of having thyroid cancer.

[64] As described above, the therapeutic method of the present invention may be performed to increase the temperature of the magnetic nanoparticles binding to thyroid cancer cells by irradiation under an alternating magnetic field, thereby making small holes on the surface of thyroid cancer cells, leading to cell death.

[65] The magnetic nanocomposite of the present invention is conjugated with a thyroid cancer-specific antibody or ligand so as to perform thermotherapy under an alternating magnetic field. Therefore, when it is used in a magnetic resonance imaging system capable of producing an alternating magnetic field adjusted for therapeutic purposes or an alternating magnetic field-generating apparatus installable on the conventional magnetic resonance imaging system, thermotherapy can be performed on the lesions immediately after diagnosis, thereby proving a new concept of simultaneous diagnosis and therapy for thyroid cancer. In addition, when there are technical difficulties in operating the alternating magnetic field-generating apparatus in the magnetic resonance imaging units, a patient can be transferred after diagnosis, and then treated with alternating magnetic fields. These effects make it possible to overcome the drawbacks and side-effects of the radioactive iodine therapy or radioactive therapy that is performed additionally for thyroidectomy patients or singly for distant metastasis, which will ease the pain of thyroid cancer patients.

[66]

Mode for the Invention

[67] Hereinafter, the present invention will be described in more detail with reference to Examples. However, these Examples are for illustrative purposes only, and the invention is not intended to be limited thereby.

[68]

[69] **Example 1: Synthesis of magnetic nanoparticles (MNPs)**

[70] A metal-oleate complex was prepared by reacting metal chloride and sodium oleate. In the typical synthesis of an iron-oleate complex, 10.8 g of iron chloride ($FeCl_3 \cdot 6H_2O$, 40 mmol, Aldrich, 98%) and 36.5 g of sodium oleate (120 mmol, TCI, 95%) were dissolved in a solvent mixture composed of 80 ml of ethanol, 60 ml of distilled water and 140 ml of hexane. The resulting solution was heated at 70°C for four hours. When the reaction was completed, the upper organic layer containing the iron-oleate complex was washed three times with 30 ml of distilled water in a separatory funnel. After washing, the hexane was evaporated to obtain an iron-oleate complex in a waxy solid form.

[71] The following is a typical synthetic procedure for approximately 12 nm monodispersed iron oxide (magnetite) nanoparticles. A total of 36 g (40 mmol) of the iron-oleate complex and 5.7 g of oleic acid (20 mmol, Aldrich, 90%) were dissolved in 200 g of 1-octadecene (Aldrich, 90%) at room temperature. The reaction mixture was heated to 320°C by elevating the temperature at a constant heating rate of 3.3°C/min, and the temperature was maintained at 320°C for 30 min. When the reaction temperature reached 320°C, a severe reaction occurred and the initial transparent solution became turbid and brownish black. The resulting solution containing the MNP was then cooled to room temperature, and 500 ml of ethanol was added to the solution to precipitate the nanoparticles. The final product of nanoparticles was obtained by centrifugation (15000 rpm).

[72]

[73] **Example 2: Synthesis of PEGylated MNP**

[74] The resulting MNPs dispersed in chloroform were then encapsulated with a PEG-phospholipid shell to endow them with biocompatibility. Typically, 2 ml of the organic dispersible 12-nm MNP in $CHCl_3$ (5 mg/ml) was mixed with 1 ml of $CHCl_3$ containing 20 mg of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000] (mPEG-2000 PE, Avanti Polar Lipids, Inc) and 10 mg of 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (DSPE-PEG(2000) Carboxylic Acid Avanti Polar Lipids, Inc.). After evaporating the solvent, the sample was incubated at 80°C under vacuum for 1 hour. The addition of 5 ml of water resulted in a clear, dark brown suspension. After filtration, the excess mPEG-2000 PE was removed by centrifugation.

[75]

[76] **Example 3: Synthesis of antibody-conjugated magnetic nanoparticles (MNPs)**

[77] To conjugate anti-TSHR antibody/anti-NIS antibody with the PEGylated MNPs, 1 ㎎ of anti-TSHR antibody/anti-NIS antibody and 2 ㎎ of PEGylated MSNPs were mixed. Then, 10 mmol of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and N-hydroxysuccinimide was added to the solution at 4℃. After reacting for 24 hours, the unwanted residues were eliminated by centrifugation.

[78]

[79] **Example 4: Cell culture**

[80] A normal rat thyroid cell, FRTL5 was provided by Dr. Do-Joon Park, Seoul National University Hospital. The cell was maintained according to the previously reported method (Ambesi-Impiombato F S; Parks L A; Coon H G, (1980) Culture of hormone-dependent functional epithelial cells from rat thyroids. Proceedings of the National Academy of Sciences of the United States of America, 77(6):3455-9., US Patent No. 4608341) with minor modifications. Briefly, the cells were cultured in advanced DMEM: Coon's F12 media (1:1 mixture; Invitrogen, Carlsbad, CA, USA) containing 5% FBS (Fetal bovine serum) and 6 types of hormones (10 mU/㎖ TSH (thyroid-stimulating hormone), 5 ㎍/㎖ insulin, 5 ㎍/㎖ transferrin, 10 ng/㎖ somatostatin, 10 ng/㎖ GHK acetate, and 0.4 ng/㎖ hydrocortisone (all these hormones were purchased from Sigma; St. Louis, MO, USA). 2 mM L-glutamine was added to the media.

[81] Nthy-ori 3.1 (hereinbelow, referred to as "Nthy") is a human immortalized normal thyroid cell line, and was provided by Dr. Ki-Wook Chung, National Cancer Center. A human cervical cancer cell line HeLa expressing no endogenous NIS protein was purchased from the Korean Cell Line Bank (Seoul, Korea). Nthy and HeLa cell lines were maintained in RPMI1640 containing 10% FBS (fetal bovine serum), 100 U/㎖ penicillin and 100 ㎍/㎖ streptomycin. HeLa-NIS, a stably transfected HeLa cell line overexpressing human NIS protein (conjugated to td Tomato protein) on a CMV promoter, was provided by professor June-Key Chung (Seoul National University, Seoul, Korea). The cell lines were cultured in RPMI1640 containing 10% FBS and 100 U/㎖ penicillin and 100 ㎍/㎖ streptomycin. All cell lines were incubated in a humidified cell culture incubator with 5% carbon dioxide supplied. If necessary, cells were treated with trypsin-EDTA (Invitrogen, Carlsbad, CA, USA) and counted using a hemocytometer.

[82]

[83] **Example 5: Flow cytometry**

[84] For flow cytometry of a cell surface antigen, NIS (sodium-iodine symporter) or TSHR (thyroid stimulating hormone receptor), the cultured cells of Example 4 were

detached from a culture plate using a cell scraper when the confluence reached 70-80%. Cells were prepared at a density of 1.0 x 10^6 per each sample, and treated with rabbit polyclonal anti-NIS antibody (Abbiotec, San Diego, CA, USA; Cat# 250552) or rabbit polyclonal anti-TSHR antibody (Abbiotec, San Diego, CA, USA; Cat# 250898) in PBS containing 3% BSA at 4℃ for 1 hour. When specifically indicated, the samples were treated with primary antibody-conjugated Fe3O4 nanoparticles by the same flow cytometry. Centrifugation was performed to obtain the cells. The cells were reacted with secondary antibody (PE-conjugated anti-rabbit IgG) diluted with PBS containing 3% BSA in a dark room at 4℃ for 30 minutes. After the additional washing step, the cells were analyzed using a FACS Calibur (BD biosciences, San Jose, CA, USA).

[85]

[86] As a result, 59.0% expression of the thyroid cell surface antigen, NIS and 50.3% expression of TSHR were observed in the normal rat thyroid cell, FRTL5 (FIG. 3), and 51.2% expression of the thyroid cell surface antigen, NIS and 22.9% expression of TSHR were observed in a human immortalized normal thyroid cell line, Nthy (FIG. 4). On the contrary, 4.7% expression of NIS and 6.9% expression of TSHR were observed in the human cervical cancer cell line, HeLa. These results indicate that NIS and TSHR are thyroid-specific antigens (FIG. 5).

[87] In addition, the binding capability of magnetic nanoparticles conjugated with the thyroid-specific antibodies, NIS and TSHR antibodies was examined in the Nthy cell line. As a result, they were found to specifically bind to the cell line (FIG. 6). These results support that the magnetic nanoparticles conjugated with the thyroid-specific antibody specifically bind to the thyroid cells, and in particular, NIS can be used in diagnosis and treatment, owing to its high binding capability.

[88]

[89] **Example 6: Immunofluorescence**

[90] For the immunofluorescent staining of the cell surface antigen NIS, HeLa and HeLa-NIS cell lines were grown on sterilized cover glass (Marienfeld, Lauda-Konlgshofen, Germany). The cell lines were fixed with 4% paraformaldehyde for 10 minutes, and non-specific binding was blocked with 1% BSA in PBS for 30 minutes. Primary antibody (rabbit IgG, rabbit polyclonal anti-NIS antibody, or NIS antibody conjugated to MNP) was diluted in a blocking solution and the fixed cells were stained for 1 hour (Rabbit polyclonal anti-NIS antibody; Abbiotec, San Diego, CA, USA). After the washing step, the cells were incubated with secondary antibody conjugated to Alexa Fluor 488 for 1 hour (Invitrogen, Carlsbad, CA, USA). After additional washing steps, the cells were mounted on a slide glass using Vectashield mounting medium with DAPI (Vector, Burlingame, CA, USA). These cells were observed on an LSM 5 Pascal laser confocal microscope (Carl Zeiss, Jena, Germany).

[91]

[92] As a result, immunofluorescence data revealed that HeLa cells were negative for NIS but HeLa-NIS cells were positive, as shown by the expression of tdTomato protein conjugated to the NIS protein. NIS-MNP, which was the sample with magnetite nanoparticles conjugated to NIS antibodies, showed a similar staining pattern to that of the anti-NIS antibodies in NIS-expressing HeLa-NIS cells. The cell morphology remained normal after exposure to the nanoparticles. These data show that NIS-MNPs bind to NIS-expressing cells in a manner similar to anti-NIS antibodies (FIG. 7).

[93] These results support that the magnetic nanocomposite binds to NIS-overexpressing HeLa cell line with a specificity like that of the anti-NIS antibody, and does not cause deformation of the magnetic nanocomposite-treated cells, thereby being capable of being used in the diagnosis and treatment safely.

[94]

[95] **Example 7: Temperature measurement in an alternating magnetic field**

[96] The heating system consists of four main subsystems; a variable frequency and amplitude sine wave function generator (15 MHz/20 Vp-p, G5100, USA), a power amplifier (DC 10 MHz/142 Vp-p, HAS4101, USA), an induction coil (length: 30 m, turns: 43, diameter: 172 ㎜, height: 320 ㎜) and an oscilloscope (Goldstar, Korea), a temperature controller (1.4-1000 K, Lake shore, USA).

[97] Magnetite nanoparticles were prepared as described above. Magnetite nanoparticles were diluted in RPMI1640 to a concentration of 7 ㎎/㎖. After the magnetic field was changed, temperature change of the solution was measured using a thermocouple (Lakeshore, Westerville, OH, USA).

[98] All data were compiled from a minimum of three replicate experiments. Data from the statistical analysis was expressed as the mean ± the standard error. The treated and control cells were compared using Student's t-test. A p-value less than 0.05 was considered statistically significant.

[99]

[100] As a result, there was a significant thermal increase in the solution containing magnetite nanoparticles after AMF irradiation, as compared to the RPMI media control group (FIG. 8).

[101] These results support that the magnetic nanoparticles of the present invention show thermotherapeutic effects, and thus specific binding of the thyroid-specific antibody-conjugated magnetic nanoparticles to thyroid cancer allows diagnosis, and the thermal increase of magnetic nanoparticles by AMF irradiation destroys cancer cells to exert thermotherapeutic effects at the same time.

[102]

[103] **Example 8: In vitro NIS-MNP induced-hyperthermia**

[104] HeLa and HeLa-NIS cells were seeded in 24-well plates. When these cells reached 70-80% confluence, the media was changed to media with or without 0.2 ㎎/㎖ NIS-MNP. After 4 hrs incubation in a humidified CO_2 incubator, the cells were harvested using a cell dissociation buffer (Invitrogen, Carlsbad, CA, USA). After washing in PBS, the collected pellet was resuspended in RPMI1640 media containing 10% FBS and 10 mM HEPES. Half of the resuspended cells were placed in an alternating magnetic field (AMF, 7 MHz) for 1 hour, and the other half were placed outside of the AMF as a control group. The frequency of the AMF device was manually controlled to be between 6.5 MHz to 7 MHz to ensure that the temperature of the sample did not exceed 41℃, because the AMF device induces non-specific cell death at 42℃ or higher. After exposure to AMF, the cells were seeded in 96-well plates and after 24-hr incubation, cell viability was measured by WST assay performed according to the manufacturer's protocol (Takara, Otsu, Japan). Briefly, the cells in the 96-well plates were treated with media containing WST-1 (Takara, Otsu, Japan) for 3 hours. During this incubation period, viable cells converted WST-1 to a water-soluble formazan dye. Quantification of the formazan dye in the microplate was performed with an ELISA plate reader.

[105]

[106] As a result, when treated with media containing 0.2 ㎎/㎖ of NIS-MNP, HeLa-NIS cells showed reduced viability (77%) compared to HeLa cells (96%) after exposure to the AMF. Non-AMF exposed HeLa and HeLa-NIS cells served as controls. To determine whether an intrinsic susceptibility to AMF exists in HeLa-NIS cells, HeLa and HeLa-NIS cells that were not treated with NIS-MNP were also exposed to AMF. Both cell lines showed similar viability compared to the control cells not exposed to AMF (FIG. 9).

[107] These results support that treatment of the alternating magnetic field suitable for the magnetic nanocomposite of the present invention inhibits cell growth, thereby treating cancer.

[108]

[109] **Example 9: In vivo MRI imaging**

[110] All in vivo MRI studies were performed using a micro 7.0 Tesla MRI System (Bruker-Biospin) equipped with a 20-cm gradient set capable supplying up to 400mT/m in a 100-sec rise time. A birdcage coil (70 mm i.d., Bruker-Biospin) was used for excitation, and an actively decoupled phased array coil was used for receiving the signal. Mice (BALB/C-nu) were anesthetized with 1.5% isoflurane in air/O2 (2:1) administered via an MR-compatible mobile inhalation anesthesia system. The tail vein was cannulated before placing mice in the magnet, and 10 ㎖/㎏ of NIS-MNP was injected intravenously. Contrast-enhanced imaging was performed using a coronal

T2*-weighted gradient echo sequence (FLASH sequence, TR=312 ms, TE=16 ms, flip angle=30°, FOV=21×21 mm, imaging matrix=256×256, slice thickness=0.6 mm) and a T2-weighted fast spin echo sequence (RARE sequence, TR=3000 ms, TE=50 ms, FOV=21×21 mm, imaging matrix=256×256, slice thickness=0.6 mm).

[111]

[112] As a result, the thyroid gland was successfully visualized in the mouse model. Obvious enhancement of the thyroid gland was observed in T2-and T2*- weighted images 1 hour after NIS-MNP injection (FIG. 10). These results support that the metal nanocomposite of the present invention can be used for the cancer-specific diagnosis by MRI without additional contrast agents.

[113]

[114] **Example 10: In vivo retention and therapeutic effect of NIS-MNPs in carcinomas**

[115] T2-weighted MRI images were obtained to evaluate the enhancing effect and proper localization of NIS-MNP in the HeLa-NIS tumor model. Nude mice were employed to establish a tumor model. One million HeLa-NIS cells were subcutaneously injected into the thigh of each nude mouse; tumors formed after approximately 20 days. Two mice were injected with 100 ㎕ of NIS-MNP (800 mg MNP) directly into the tumors and the control group mice were injected with saline to exclude the direct effect of AMF itself on the tumor. One hour after injection of the MNPs, all mice were exposed to the AMF (frequency: 7 MHz) for one hour and were again treated with AMF 24 hours after injection (FIG. 11). Tumor size was measured six times: pre-injection, and then on days 2 through 6 post-injection. MR imaging (T2-weighted) was performed on the NIS-MNP-injected group and pre-injection group in order to evaluate the distribution and enhancing effects of the nanoparticles. After the last measurement, the mice were sacrificed and 6-㎛-thick tumor specimens were prepared for histochemical study according to the Prussian blue staining protocol to visualize ferric ion deposits, which represent remnant NIS-MNPs bound to HeLa-NIS cells.

[116]

[117] As a result, the MRI showed successful enhancement of the tumors 1 hour after NIS-MNP injection (FIG. 12). All the mice were treated with AMF irradiation immediately after MRI imaging. Two mice that were injected with NIS-MNP exhibited growth inhibition compared to the saline-injected group (FIG. 13). When the percent tumor volume was measured relative to the original volume, there was a 15% and 39% volume increase, respectively, in the NIS-MNP group and a 95% and 130% volume increase, respectively, in the control group. Histochemical review revealed residual NIS-MNPs along the peripheral subcapsular region of the tumor, whereas there was no Prussian blue staining in the control group and central portion (FIG. 14). These results

suggest that the particles were effectively removed by RES (reticular endothelial system), considering the injected amount of NIS-MNP.

[118]

[119] Taken together, the results indicate that the metal nanocomposite of the present invention effectively binds to thyroid cancer expressing NIS or TSHR, leading to enhancement of T2-weighted MR images. Moreover, when the metal nanocomposite of the present invention is directly injected into NIS-expressing tumors, an inhibitory effect of AMF on tumor growth can be observed, and the particles are safely removed from the tissue. Therefore, NIS-MNP could be a potential substitute for I-131 whole body scan and therapy.

Claims

[Claim 1] A magnetic nanocomposite, comprising
a magnetic nanoparticle;
a biocompatible polymer encapsulating the magnetic nanoparticle; and
a thyroid cancer-specific polypeptide that is conjugated to the bio-compatible polymer.

[Claim 2] The magnetic nanocomposite according to claim 1, wherein the magnetic nanoparticle has a spinel structure or an inverse spinel structure.

[Claim 3] The magnetic nanocomposite according to claim 1, wherein the magnetic nanoparticle is iron oxide or ferrite.

[Claim 4] The magnetic nanocomposite according to claim 3, wherein the iron oxide is Fe_2O_3 or Fe_3O_4, and the ferrite is $MnFe_2O_4$, $CoFe_2O_4$, $NiFe_2O_4$ or $ZnFe_2O_4$.

[Claim 5] The magnetic nanocomposite according to claim 1, wherein the magnetic nanoparticle has a diameter of 1 to 200 nm.

[Claim 6] The magnetic nanocomposite according to claim 1, wherein the bio-compatible polymer is selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, Pluronic (polyethylene glycol-polypropylene glycol-polyethylene glycol copolymer), polyetherimide (PEI), polyvinylpyrrolidone (PVP), N-isopropylacrylamide (NIPAM), and hexa-decyltrimethylammonium bromide (CTAB).

[Claim 7] The magnetic nanocomposite according to claim 1, wherein the thyroid cancer-specific polypeptide is a non-antibody or antibody capable of specifically binding to a thyroid cancer surface protein.

[Claim 8] The magnetic nanocomposite according to claim 7, wherein the thyroid cancer surface protein is NIS (sodium iodide symporter) or thyroid stimulating hormone receptor (TSHR).

[Claim 9] The magnetic nanocomposite according to claim 1, wherein the thyroid cancer is differentiated thyroid cancer or non-differentiated thyroid cancer.

[Claim 10] A diagnostic composition for thyroid cancer, comprising the magnetic nanocomposite of any one of claims 1 to 9.

[Claim 11] A therapeutic composition for thyroid cancer, comprising the magnetic nanocomposite of any one of claims 1 to 9.

[Claim 12] The therapeutic composition according to claim 11, wherein the

treatment of thyroid cancer is performed to destroy thyroid cancer cells by heat produced from magnetic nanocomposite by irradiation of an alternating magnetic field.

[Claim 13] A method for providing information for thyroid cancer diagnosis, comprising the steps of:

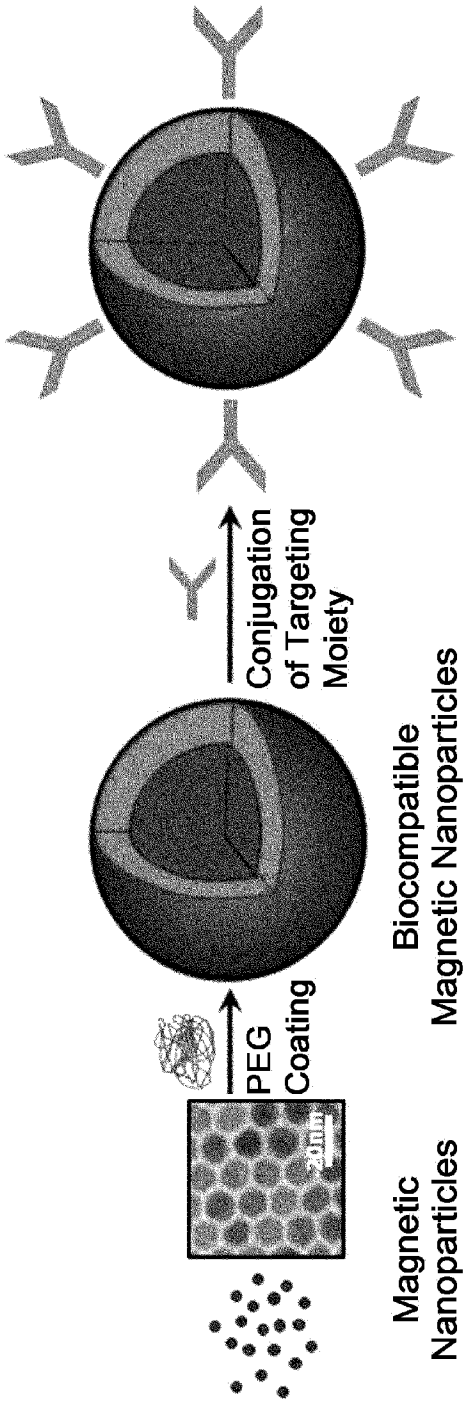
(a) administering a composition including the magnetic nanocomposite of any one of claims 1 to 9 to a patient suspected of having thyroid cancer; and

(b) detecting the presence of the magnetic nanocomposite in the patient by magnetic resonance imaging (MRI).

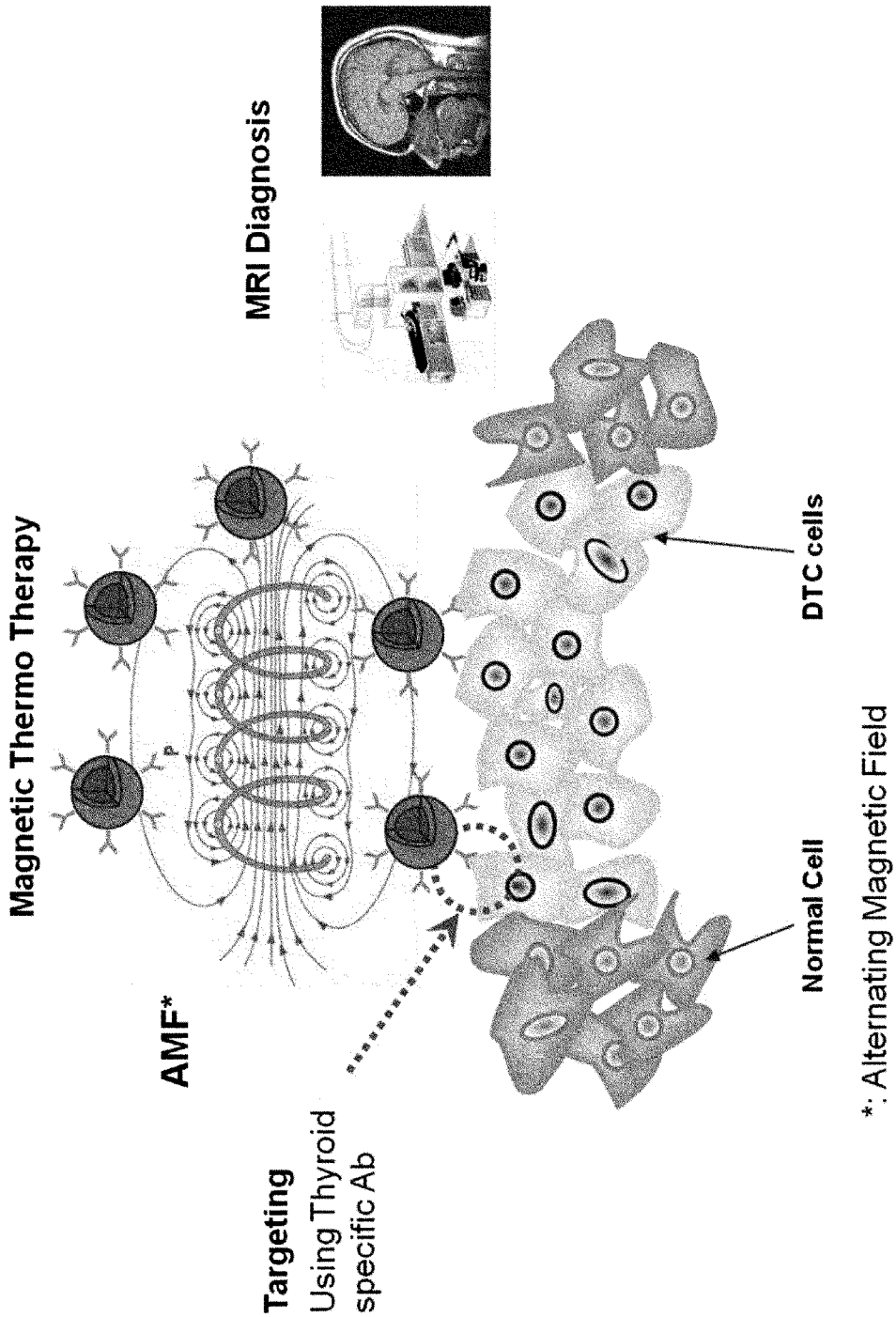
[Claim 14] A therapeutic method for thyroid cancer, comprising the steps of administering a composition including the magnetic nanocomposite of any one of claims 1 to 9 to a patient suspected of having thyroid cancer.

[Claim 15] The therapeutic method according to claim 14, further comprising the step of using an alternating magnetic field generated in an alternating magnetic field-generating apparatus.

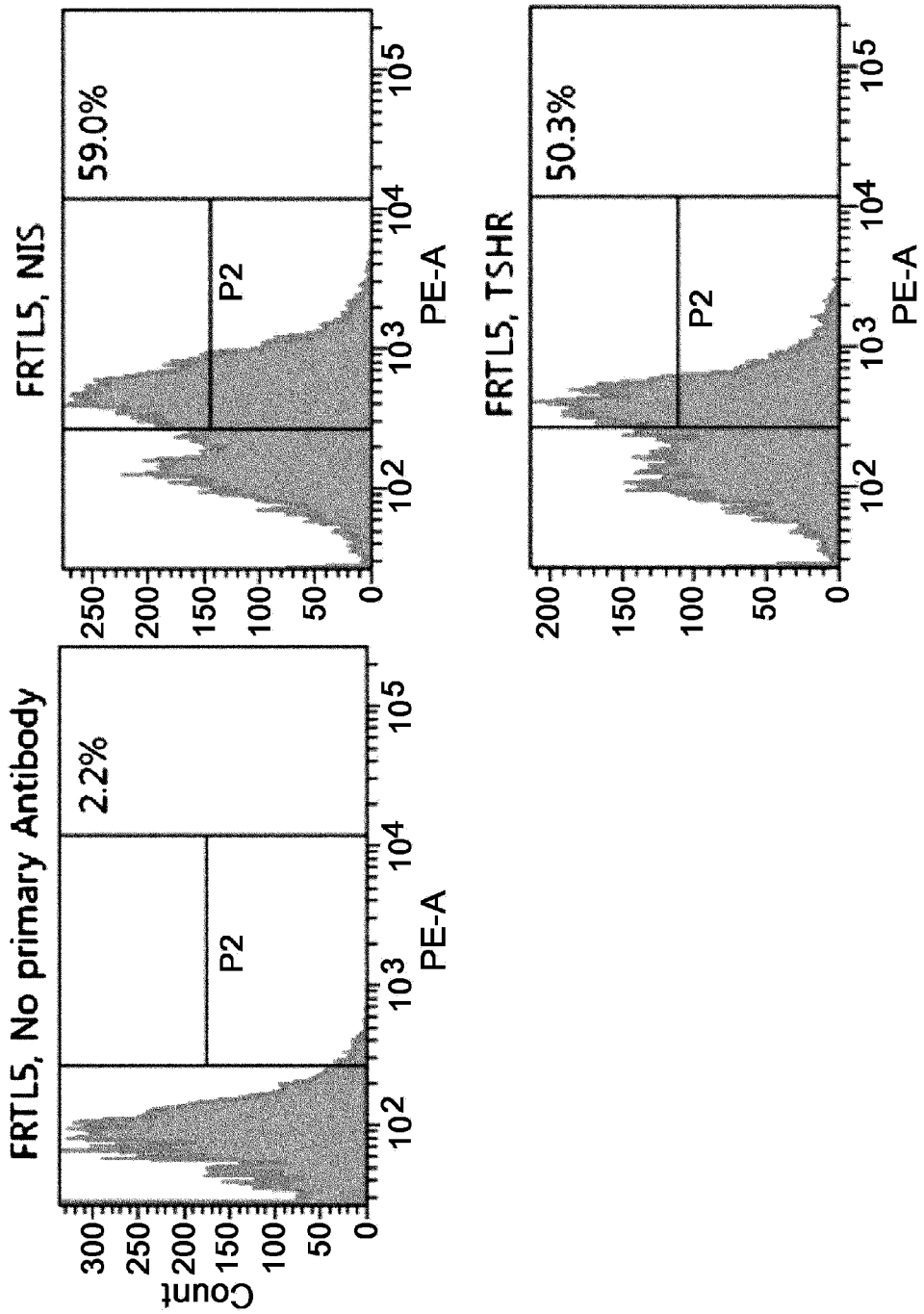
[Fig. 1]


Conjugation of Targeting Moiety
PEG Coating
Biocompatible Magnetic Nanoparticles
Magnetic Nanoparticles

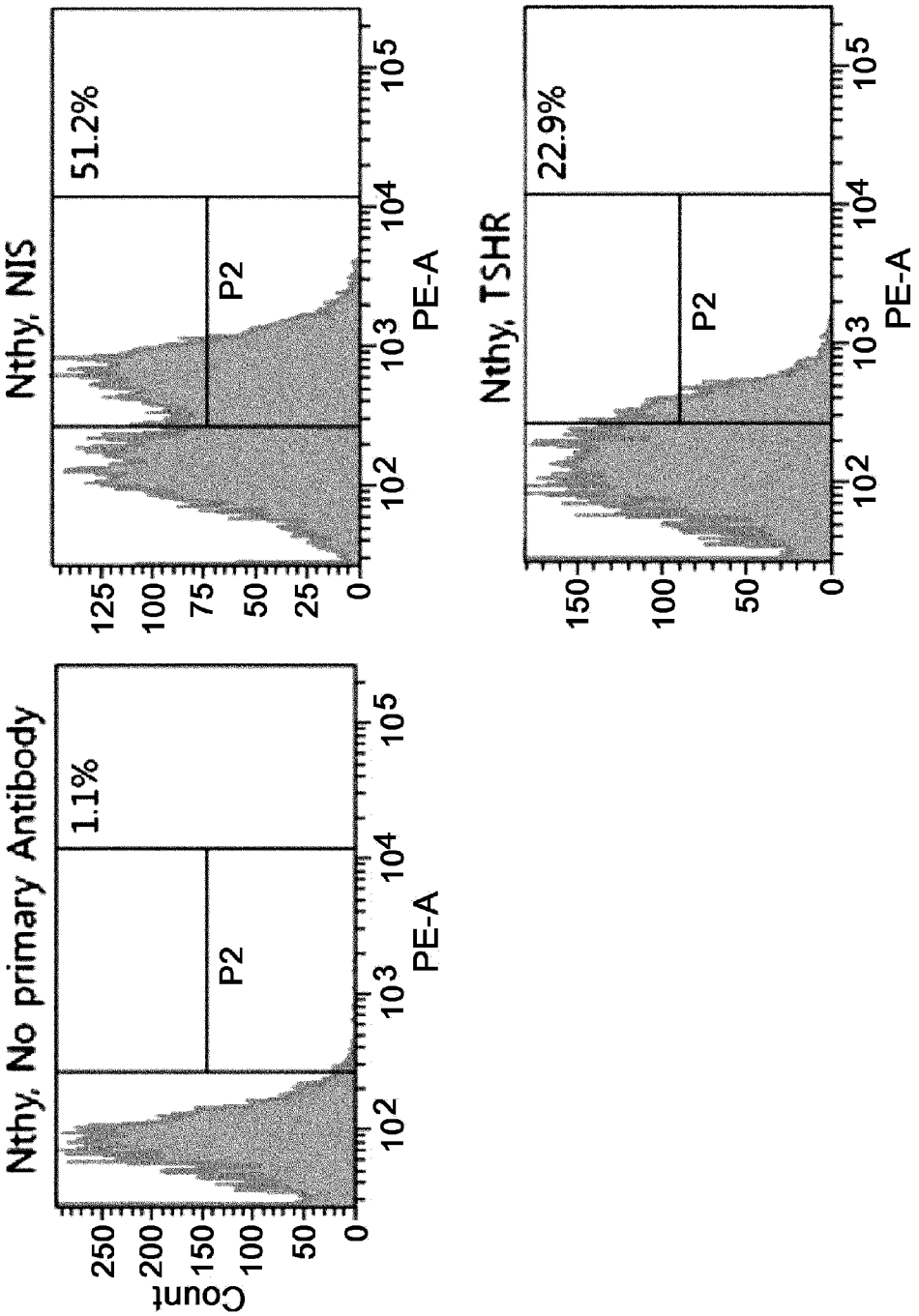
[Fig. 2]


MRI Diagnosis
Magnetic Thermo Therapy
AMF*
DTC cells
Normal Cell
Targeting
Using Thyroid specific Ab
*: Alternating Magnetic Field

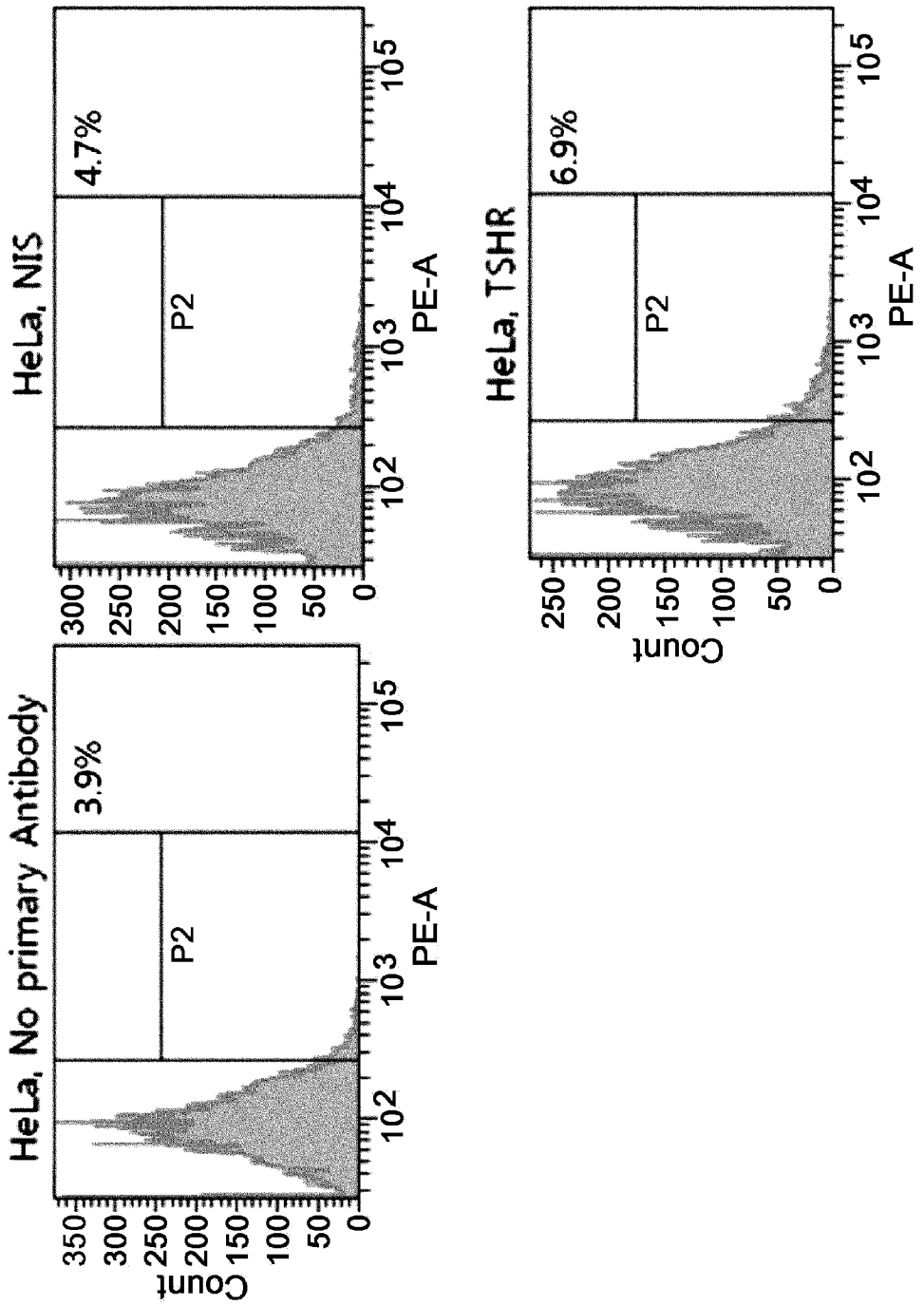
[Fig. 3]


FRTL5, No primary Antibody
2.2%
P2
FRTL5, NIS
59.0%
P2
FRTL5, TSHR
50.3%
P2
Count
PE-A
0
50
100
150
200
250
300
10^2
10^3
10^4
10^5

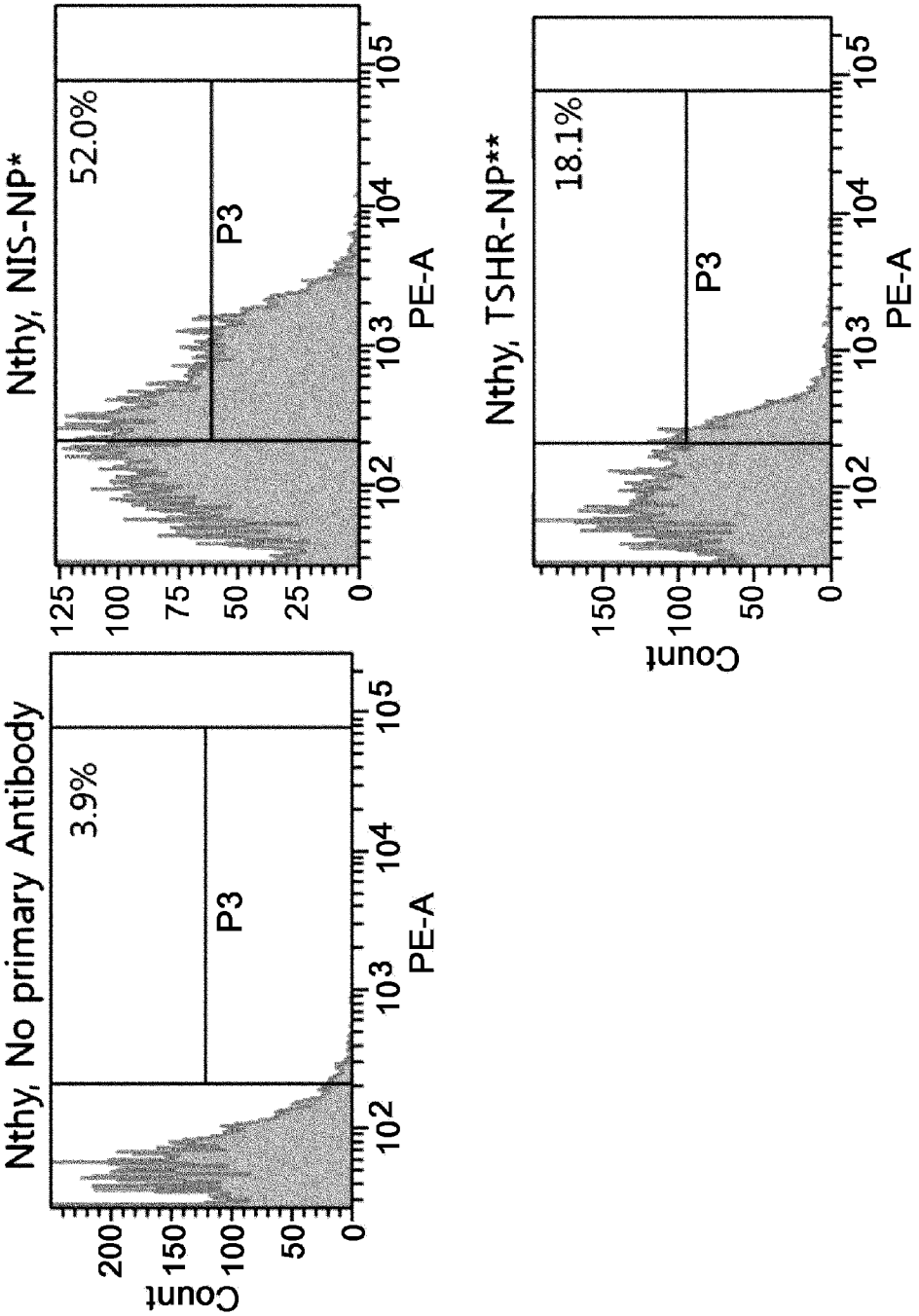
[Fig. 4]


Nthy, No primary Antibody
1.1%
P2
Count
PE-A
Nthy, NIS
51.2%
P2
PE-A
Nthy, TSHR
22.9%
P2
PE-A

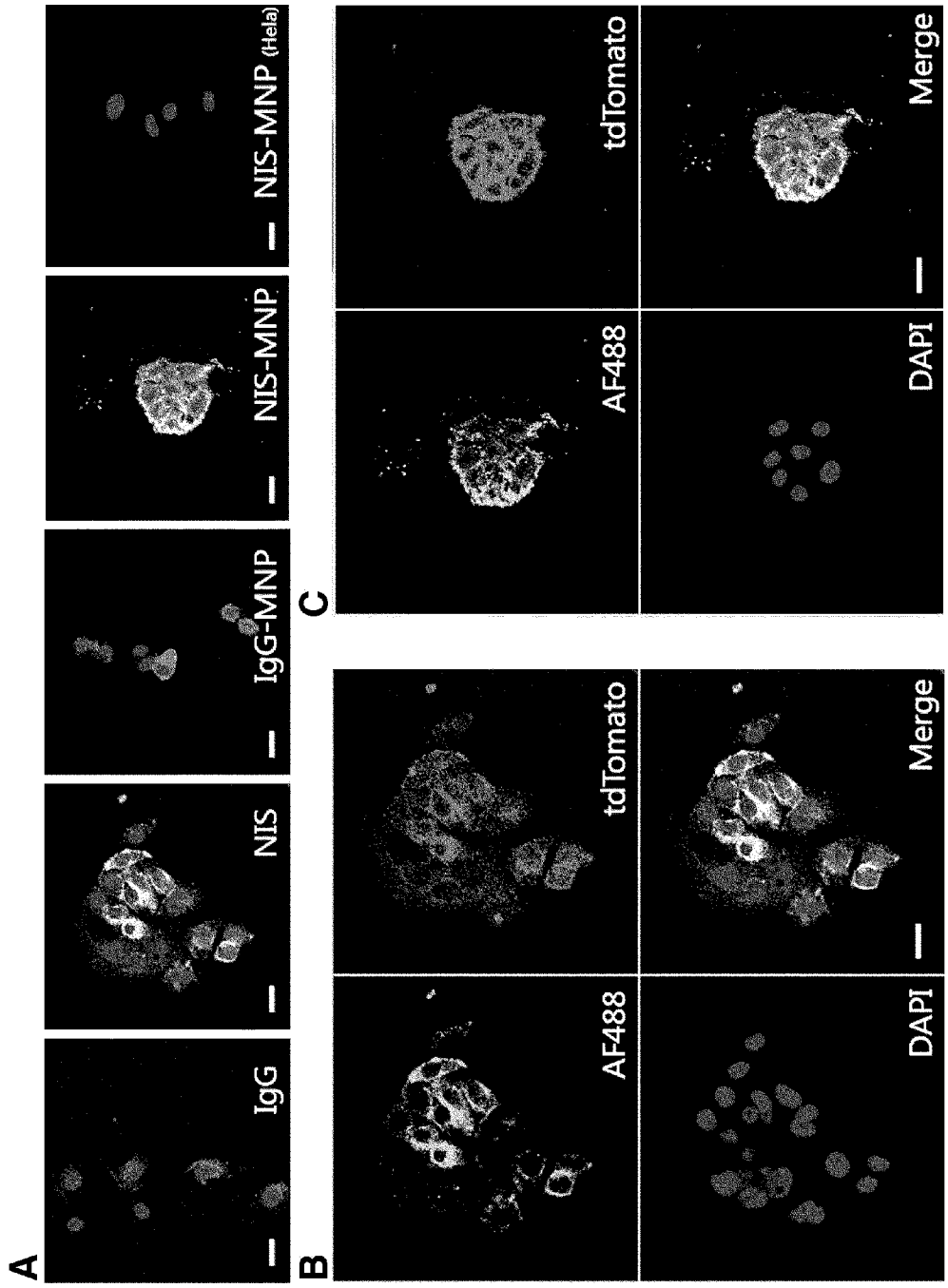
[Fig. 5]


HeLa, No primary Antibody
3.9%
P2
HeLa, NIS
4.7%
P2
HeLa, TSHR
6.9%
P2
Count
PE-A
0
50
100
150
200
250
300
350
10^2
10^3
10^4
10^5

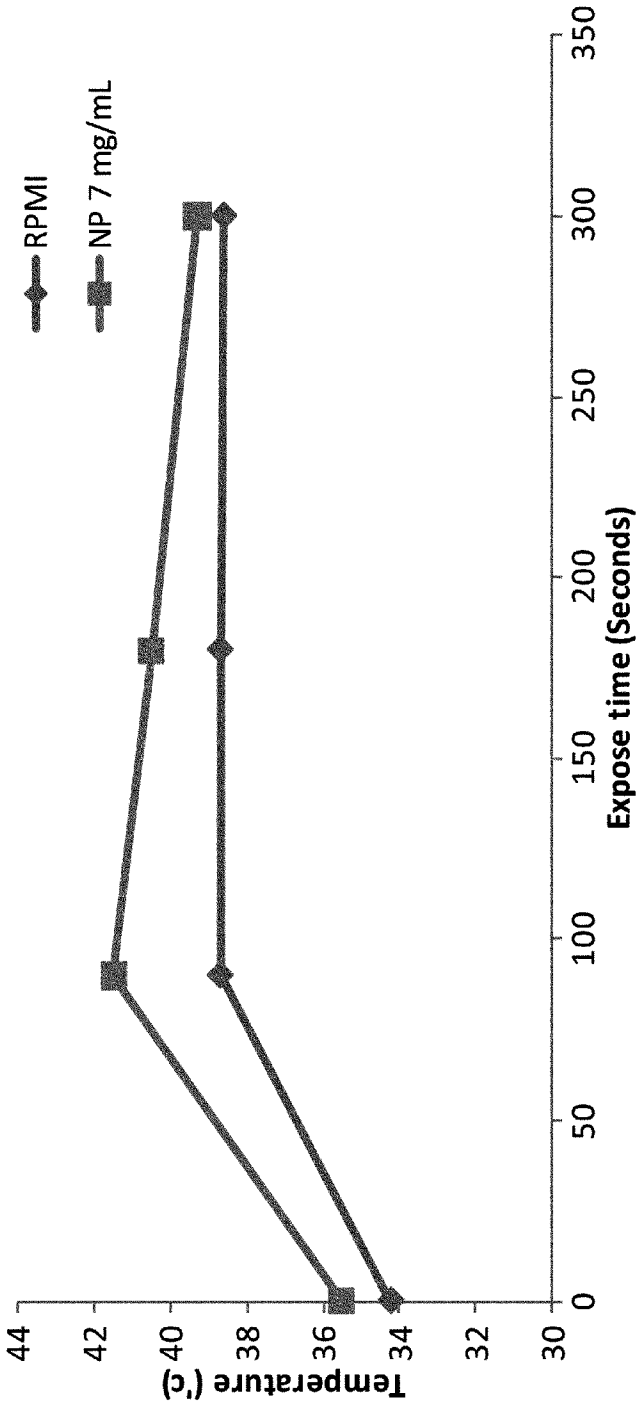
[Fig. 6]


Nthy, NIS-NP*
52.0%
P3
Nthy, TSHR-NP**
18.1%
P3
Nthy, No primary Antibody
3.9%
P3
Count
PE-A
0
25
50
75
100
125
150
200
10²
10³
10⁴
10⁵

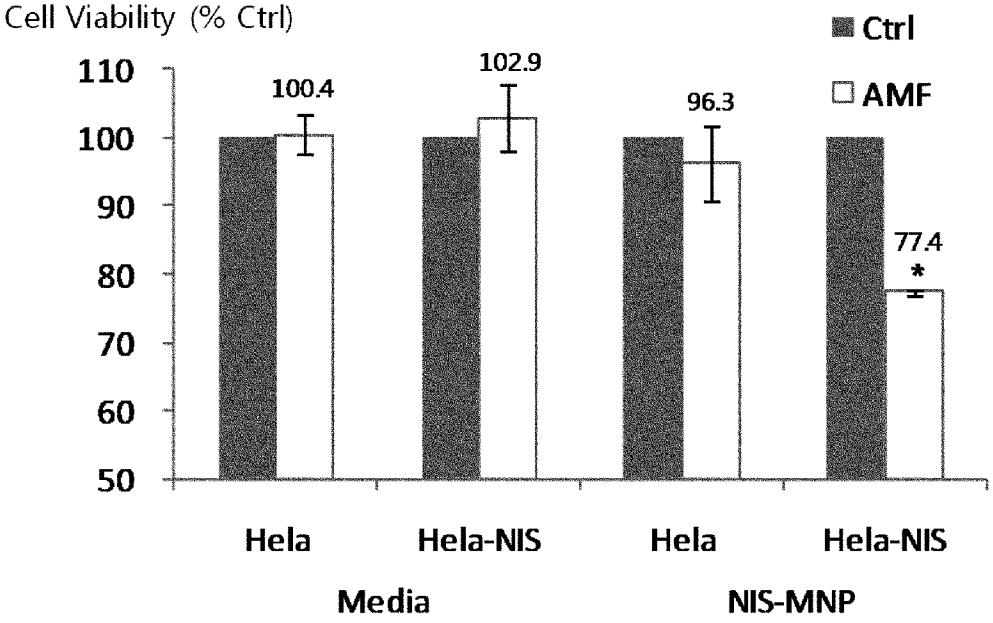
[Fig. 7]


A
IgG
NIS
IgG-MNP
NIS-MNP
NIS-MNP (Hela)
B
AF488
tdTomato
DAPI
Merge
C
AF488
tdTomato
DAPI
Merge

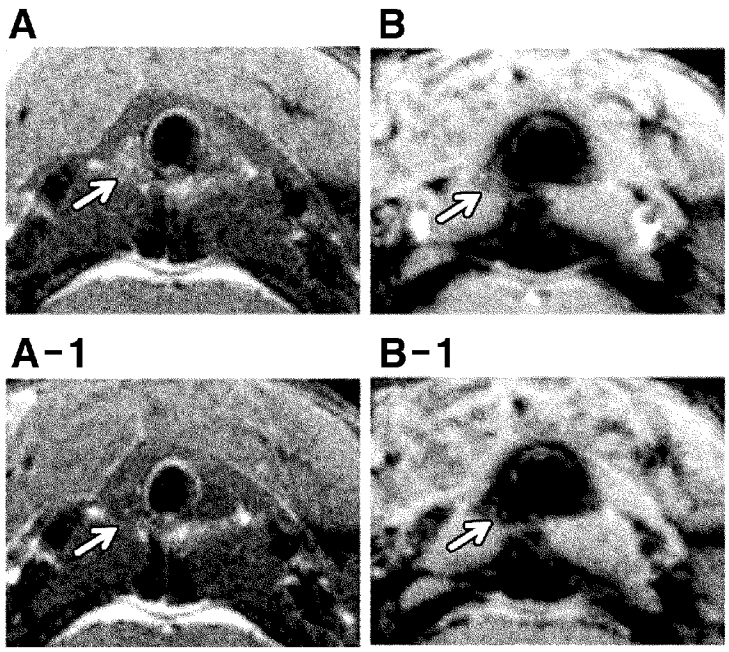
[Fig. 8]


RPMI
NP 7 mg/mL
Temperature (°c)
Expose time (Seconds)
44
42
40
38
36
34
32
30
0
50
100
150
200
250
300
350

[Fig. 9]

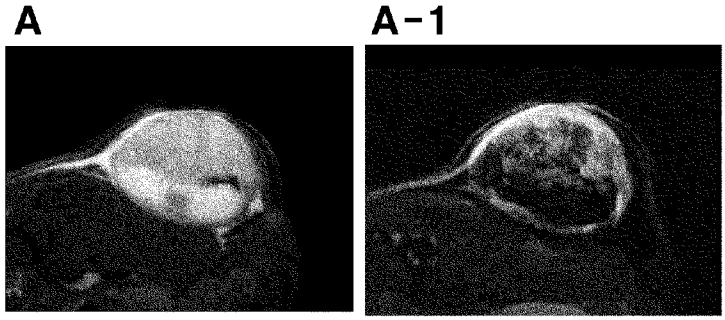

Cell Viability (% Ctrl)
Ctrl
AMF
110
100
90
80
70
60
50
100.4
102.9
96.3
77.4
*
Hela
Hela-NIS
Hela
Hela-NIS
Media
NIS-MNP

[Fig. 10]

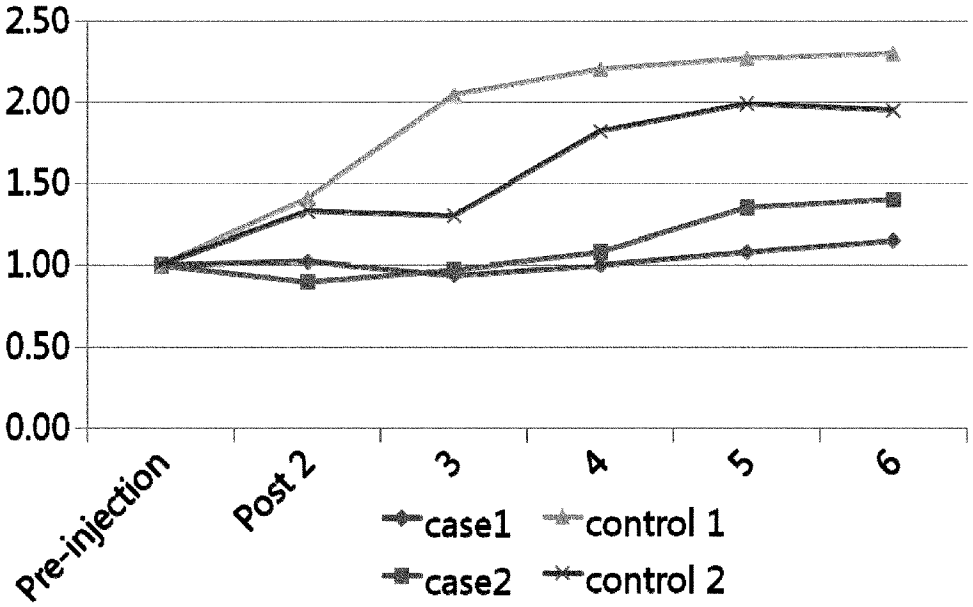

A
B
A-1
B-1

[Fig. 11]

[Fig. 12]



[Fig. 13]



[Fig. 14]