US010518096B2

(12) **United States Patent**
Esenaliev

(10) **Patent No.: US 10,518,096 B2**
(45) **Date of Patent: Dec. 31, 2019**

(54) **NONINVASIVE THERAPIES IN THE TREATMENT OF PATHOGENIC INFECTIONS**

(71) Applicant: **Rinat O. Esenaliev**, League City, TX (US)

(72) Inventor: **Rinat O. Esenaliev**, League City, TX (US)

(73) Assignee: **Board of Regents, The University of Texas System**, Austin, TX (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **15/914,866**

(22) Filed: **Mar. 7, 2018**

(65) **Prior Publication Data**

US 2018/0193657 A1 Jul. 12, 2018

Related U.S. Application Data

(63) Continuation of application No. 15/332,932, filed on Oct. 24, 2016, now Pat. No. 9,931,516, which is a continuation of application No. 12/821,398, filed on Jun. 23, 2010, now Pat. No. 9,504,824.

(60) Provisional application No. 61/322,515, filed on Apr. 9, 2010, provisional application No. 61/219,693, filed on Jun. 23, 2009.

(51) **Int. Cl.**

A61M 31/00	(2006.01)
A61N 1/40	(2006.01)
A61M 37/00	(2006.01)
A61N 2/00	(2006.01)
A61N 1/32	(2006.01)
A61N 5/02	(2006.01)
A61N 5/06	(2006.01)
A61N 1/30	(2006.01)
A61N 5/067	(2006.01)

(52) **U.S. Cl.**

CPC ***A61N 1/406*** (2013.01); ***A61M 37/0092*** (2013.01); ***A61N 1/325*** (2013.01); ***A61N 2/002*** (2013.01); ***A61N 5/025*** (2013.01); ***A61N 5/062*** (2013.01); *A61N 1/30* (2013.01); *A61N 2005/067* (2013.01); *A61N 2005/0659* (2013.01); *A61N 2005/0661* (2013.01); *A61N 2005/0662* (2013.01)

(58) **Field of Classification Search**

CPC A61M 37/0092; A61N 1/30; A61N 1/325; A61N 2/002

USPC 604/20

See application file for complete search history.

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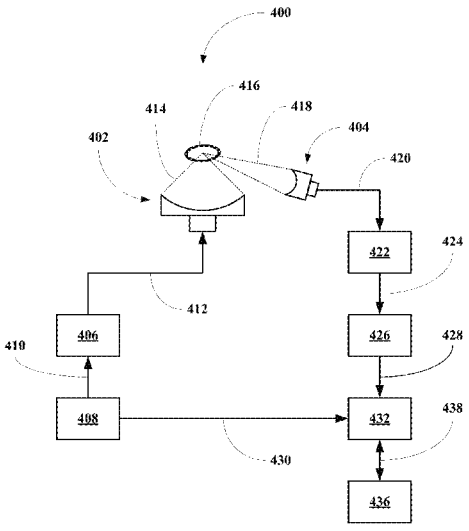
Primary Examiner — Jason E Flick

(74) *Attorney, Agent, or Firm* — Blank Rome LLP

(57) **ABSTRACT**

Methods are disclosed for treating cell components, cells, organelles, organs, and/or tissues with acoustic energy, electromagnetic energy, static or alternating electric fields, and/or static or alternating magnetic fields in the presence or absence of exogenous particulate agents for therapeutic applications.

10 Claims, 18 Drawing Sheets



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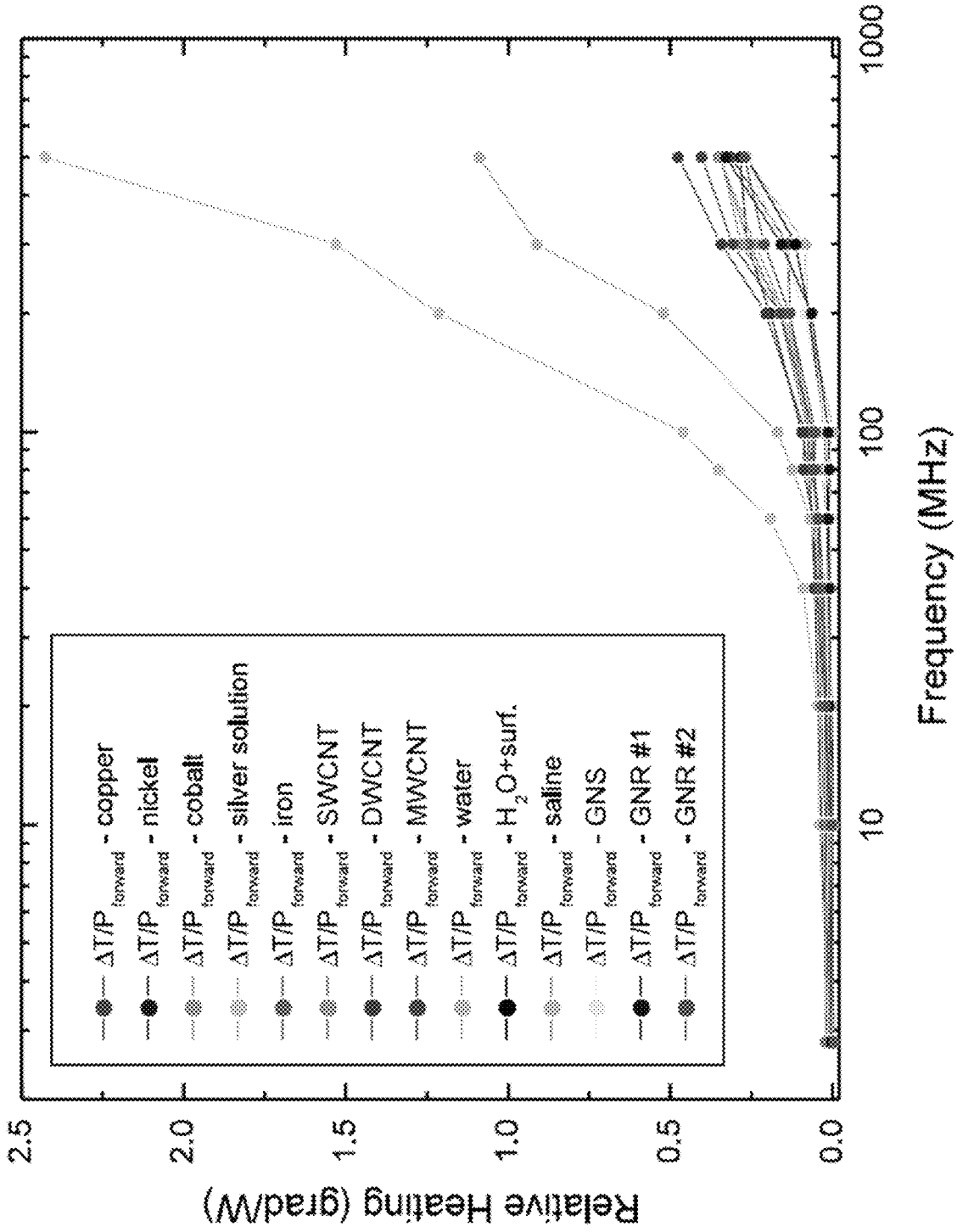
Relative Heating (grad/W)
Frequency (MHz)
2.5
2.0
1.5
1.0
0.5
0.0
10
100
1000
ΔT/P forward - copper
ΔT/P forward - nickel
ΔT/P forward - cobalt
ΔT/P forward - silver solution
ΔT/P forward - iron
ΔT/P forward - SWCNT
ΔT/P forward - DWCNT
ΔT/P forward - MWCNT
ΔT/P forward - water
ΔT/P forward - H2O+surf.
ΔT/P forward - saline
ΔT/P forward - GNS
ΔT/P forward - GNR #1
ΔT/P forward - GNR #2

FIG. 1

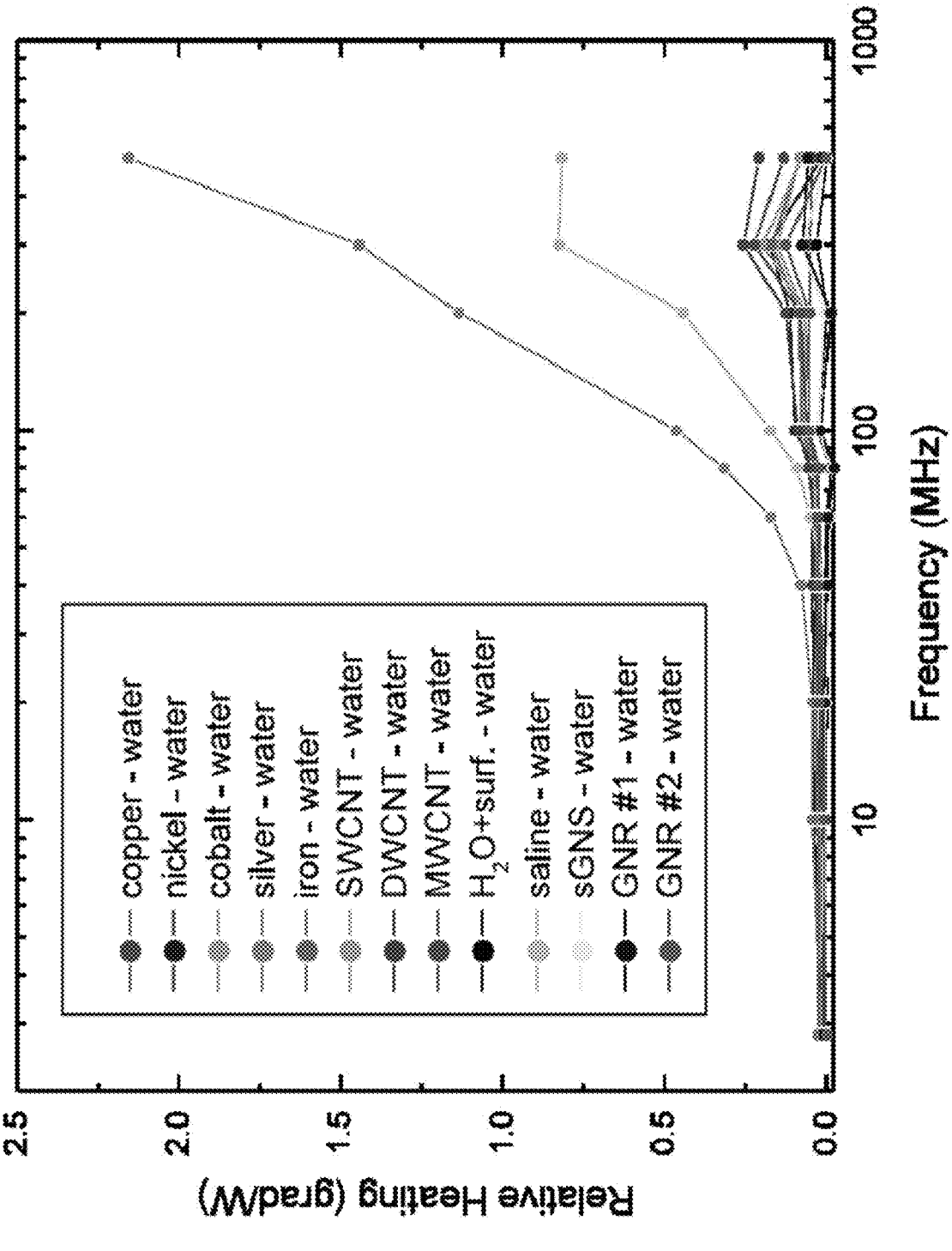
Relative Heating (grad/W)
2.5
2.0
1.5
1.0
0.5
0.0
10
100
1000
Frequency (MHz)
copper - water
nickel - water
cobalt - water
silver - water
iron - water
SWCNT - water
DWCNT - water
MWCNT - water
H_2O+surf. - water
saline - water
sGNS - water
GNR #1 - water
GNR #2 - water

Fig. 2

FIG. 3A

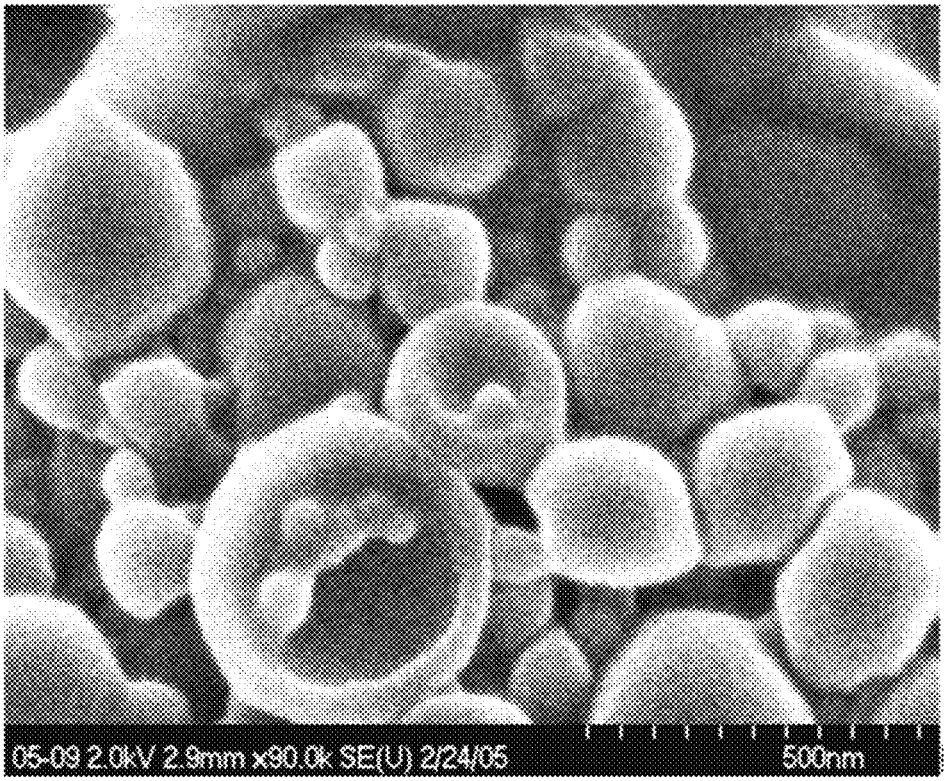
05-09 2.0kV 2.9mm x80.0k SE(U) 2/24/05
500nm

FIG. 3B

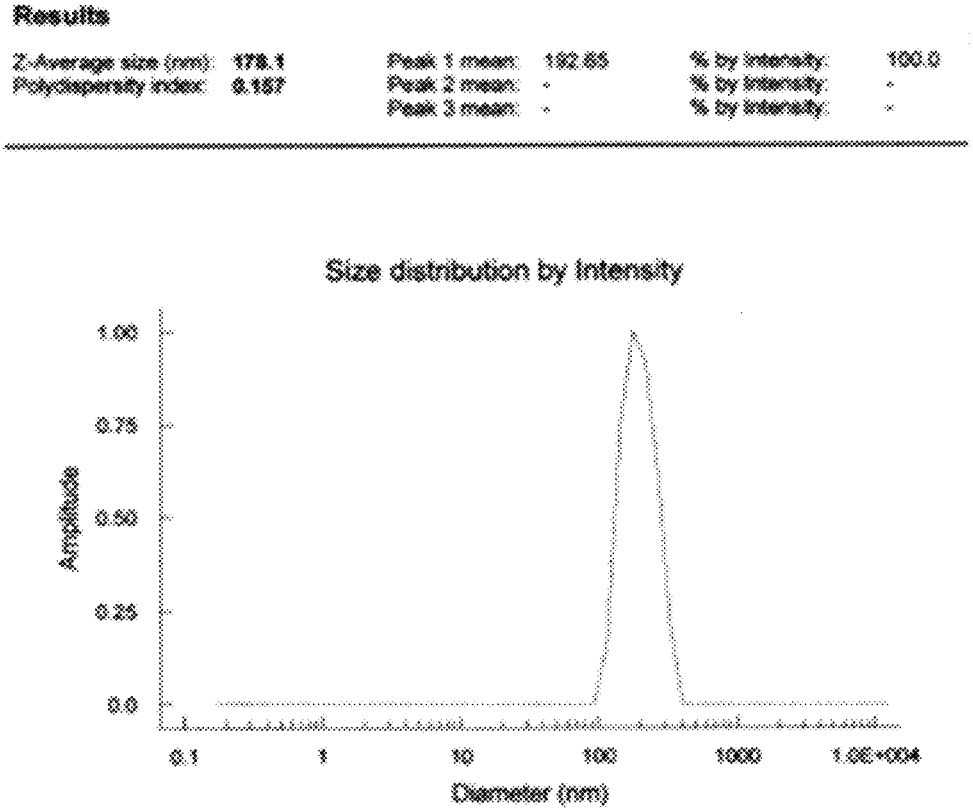
Results
Z-Average size (nm): 178.1
Polydispersity index: 0.187
Peak 1 mean: 192.65
Peak 2 mean: -
Peak 3 mean: -
% by Intensity: 100.0
% by Intensity: -
% by Intensity: -
Size distribution by Intensity
Amplitude
1.00
0.75
0.50
0.25
0.0
0.1
1
10
100
1000
1.0E+004
Diameter (nm)

Fig. 4

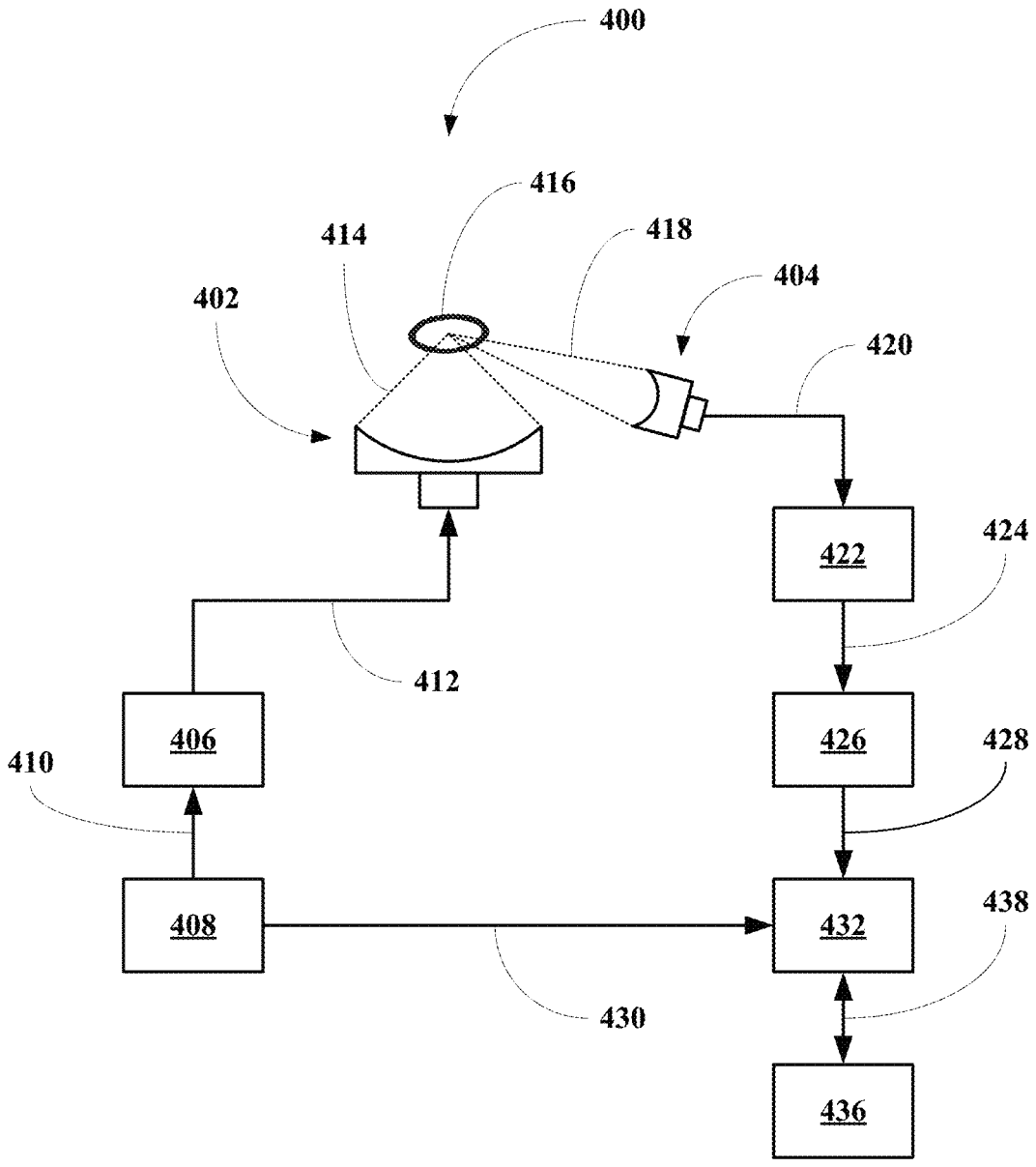
400
416
414
418
404
402
420
422
424
412
406
426
428
410
408
432
438
430
436

Fig. 5A

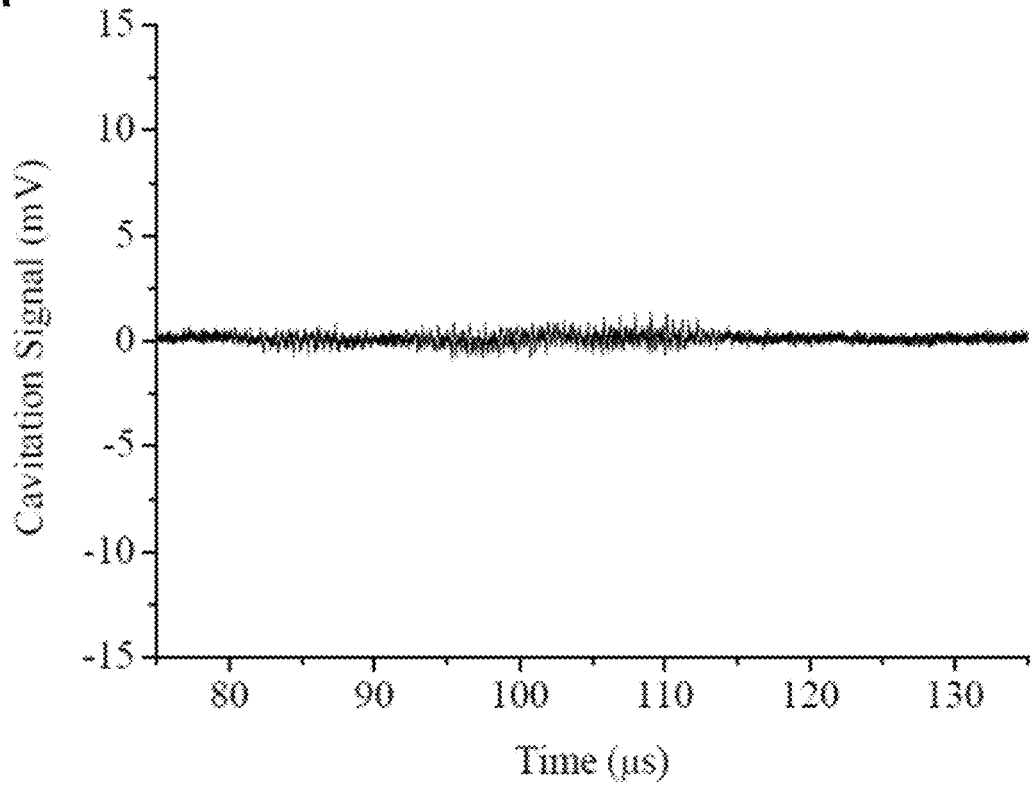
Cavitation Signal (mV)
15
10
5
0
-5
-10
-15
80
90
100
110
120
130
Time (μs)

Fig. 5B

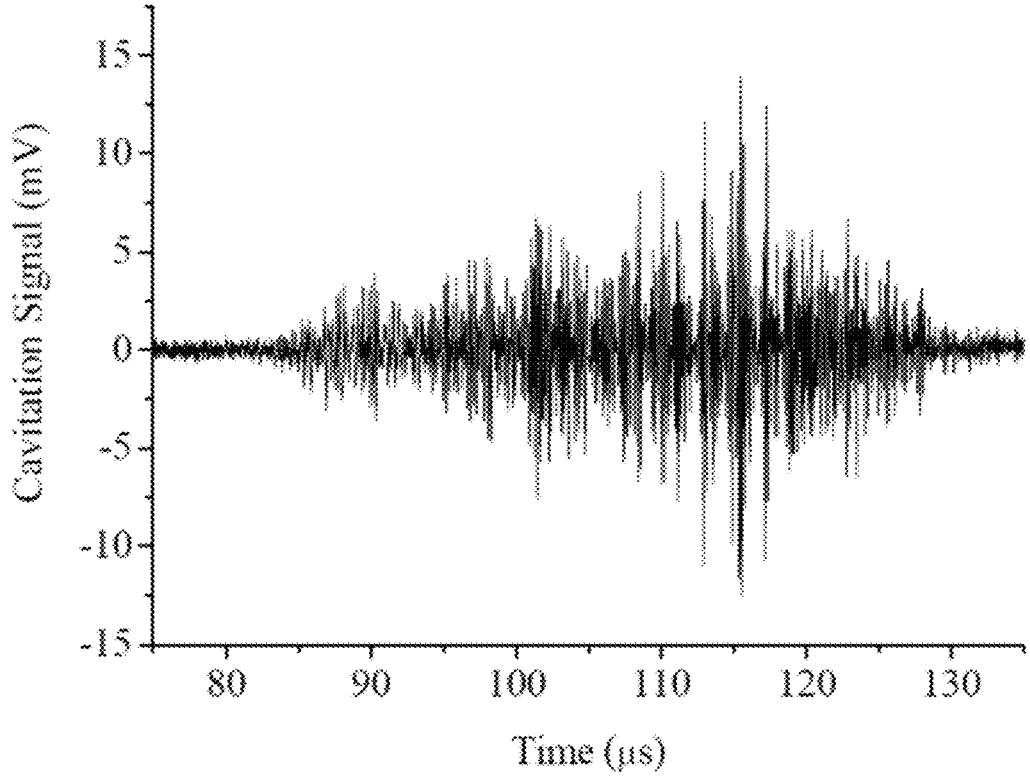
Cavitation Signal (mV)
15
10
5
0
-5
-10
-15
80
90
100
110
120
130
Time (μs)

Fig. 6A

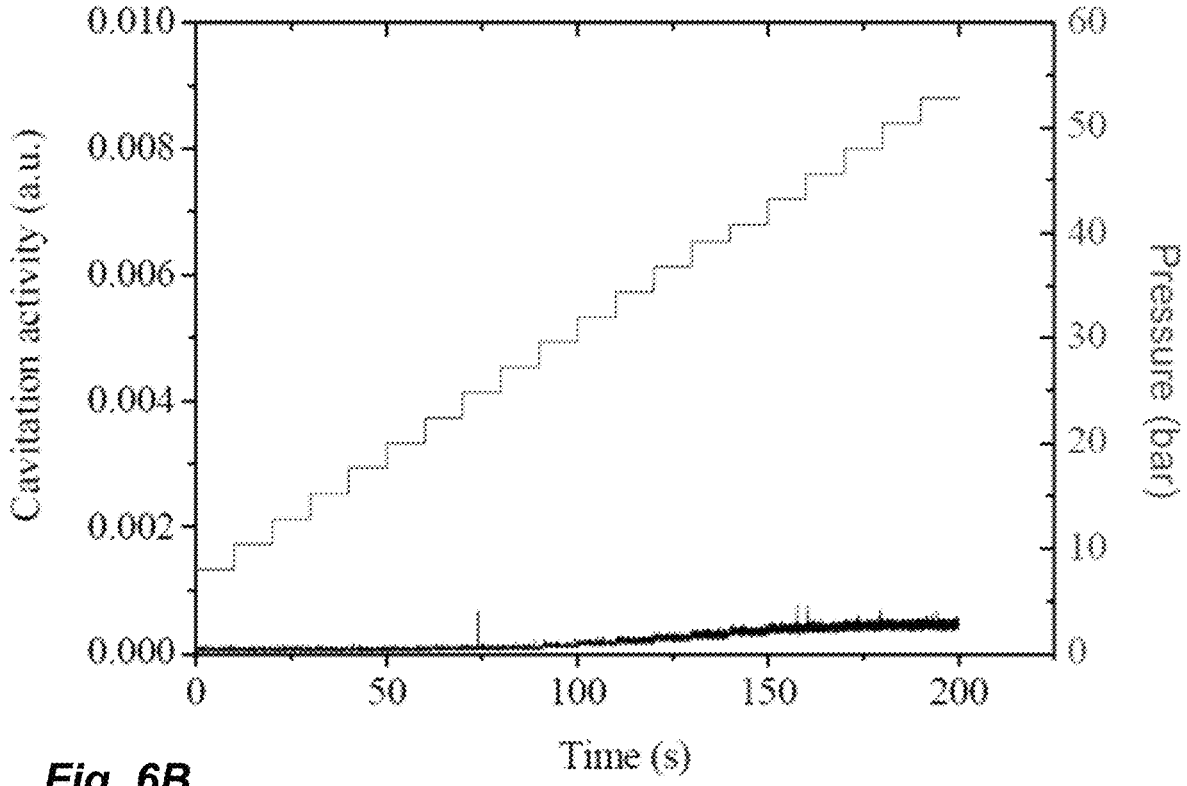
Cavitation activity (a.u.)
0.010
0.008
0.006
0.004
0.002
0.000
Pressure (bar)
60
50
40
30
20
10
0
0
50
100
150
200
Time (s)

Fig. 6B

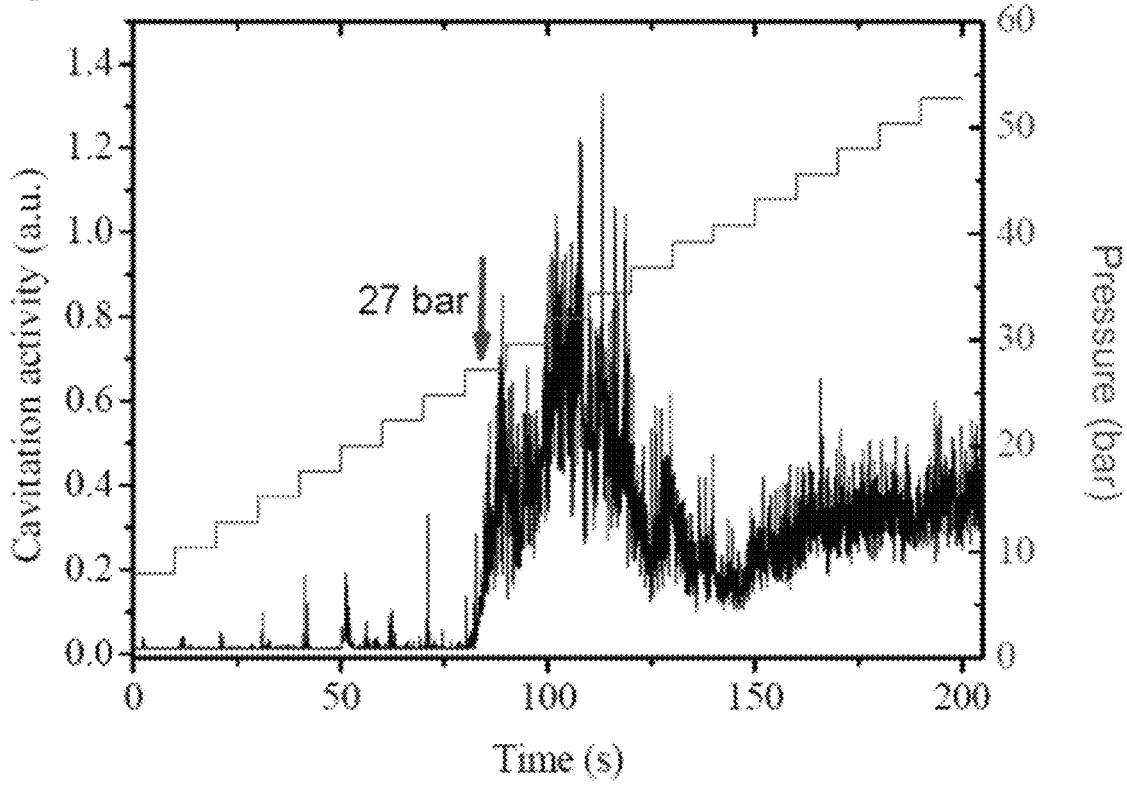
Cavitation activity (a.u.)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
27 bar
Pressure (bar)
60
50
40
30
20
10
0
0
50
100
150
200
Time (s)

Fig. 7A

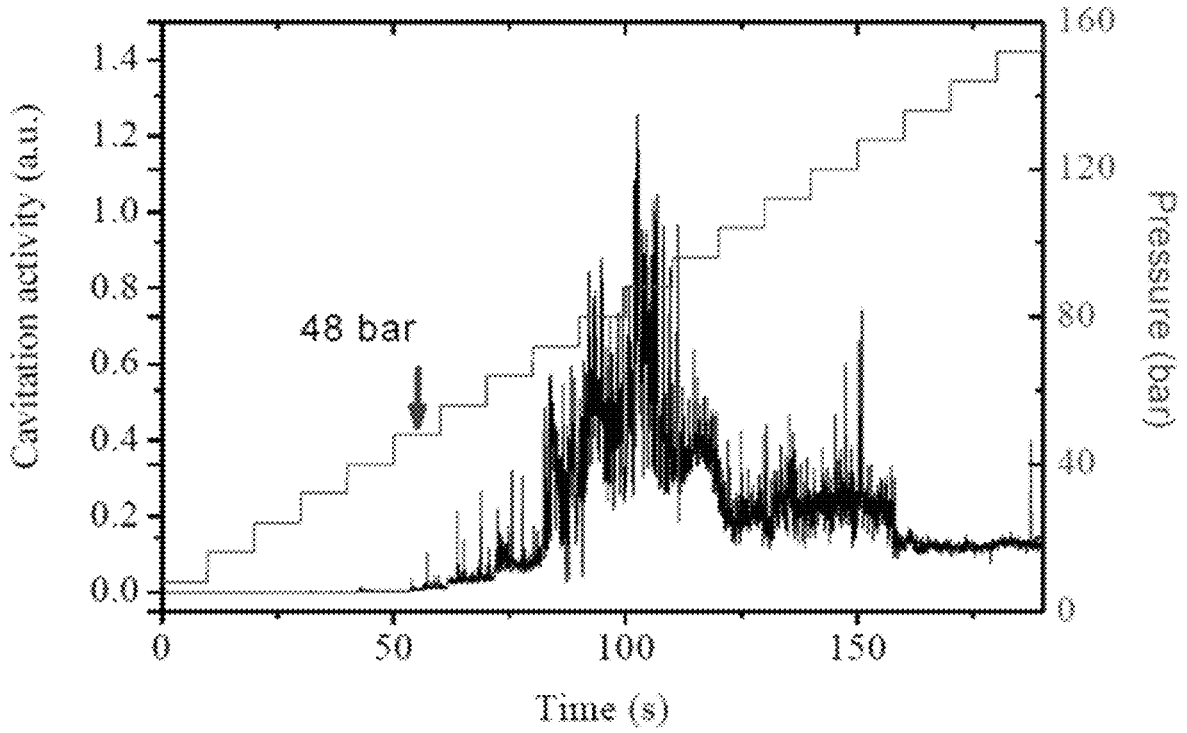
Cavitation activity (a.u.)
Pressure (bar)
Time (s)
48 bar
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
40
80
120
160
0
50
100
150

Fig. 7B

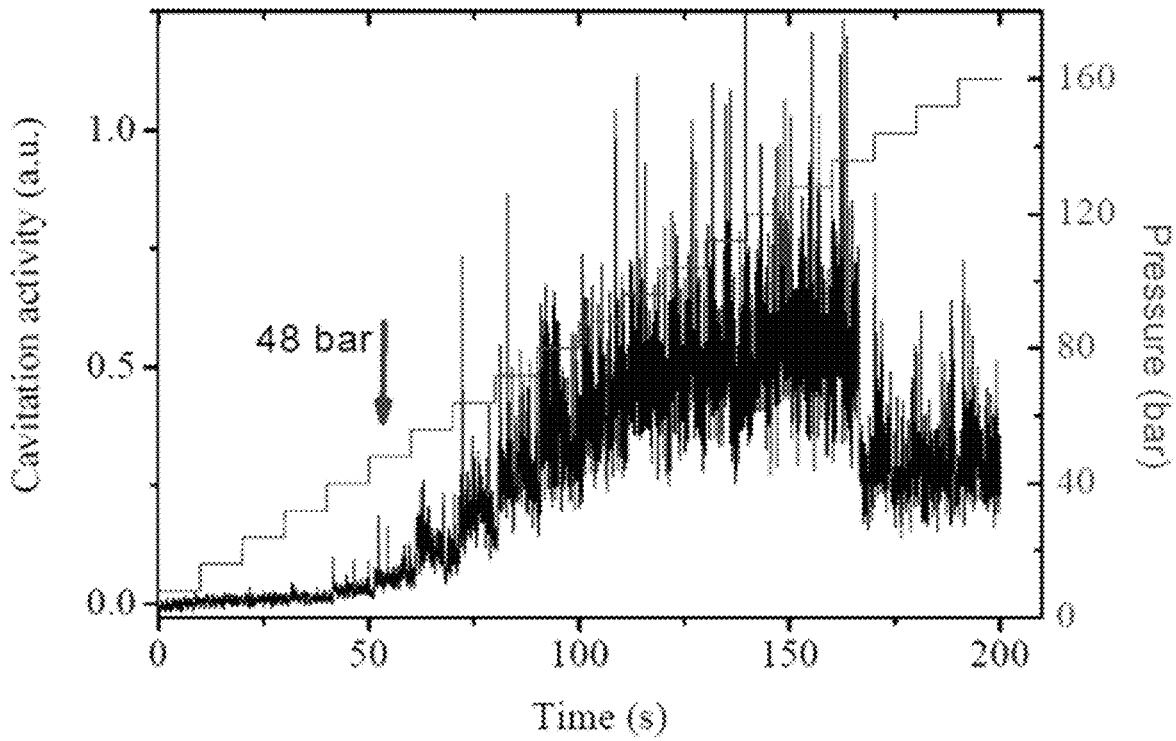
Cavitation activity (a.u.)
Pressure (bar)
Time (s)
48 bar
0.0
0.5
1.0
0
40
80
120
160
0
50
100
150
200

Fig. 8A

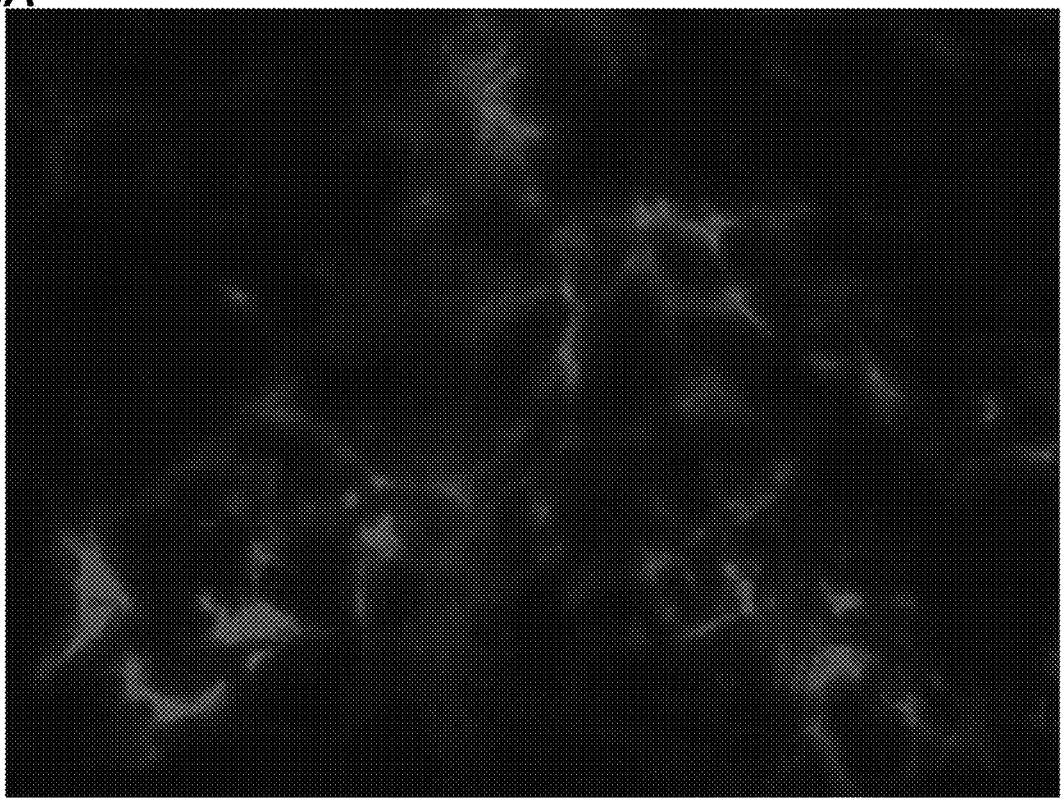

Fig. 8B

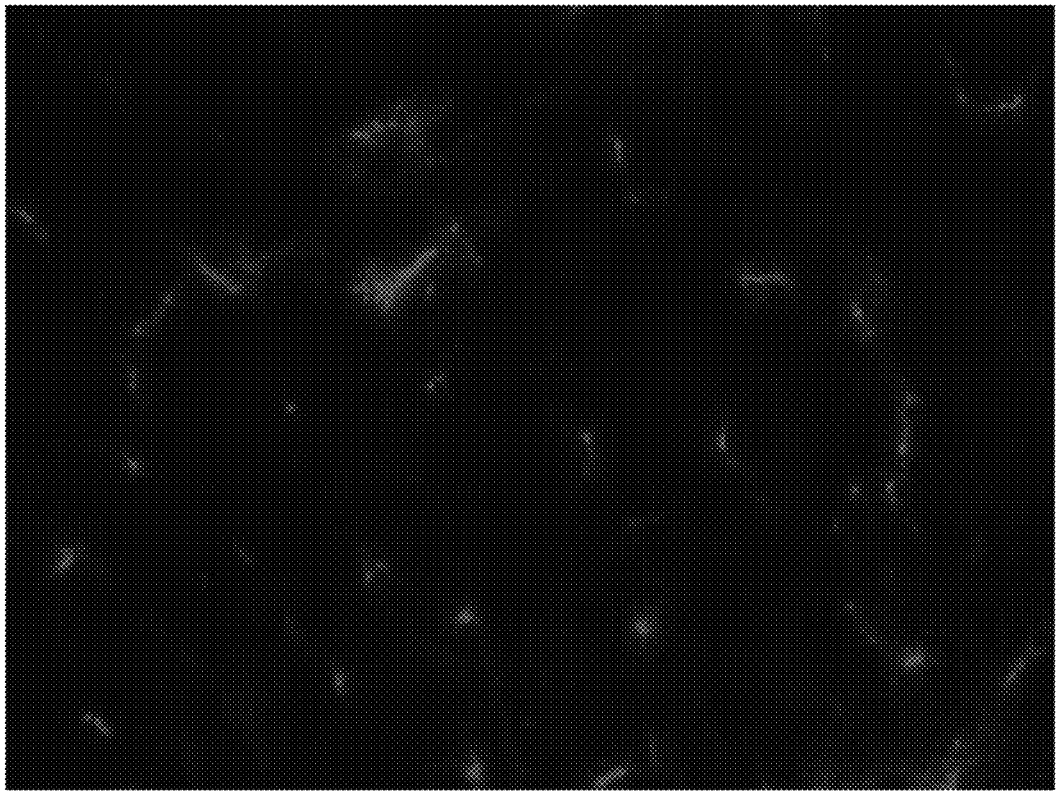

Fig. 9A

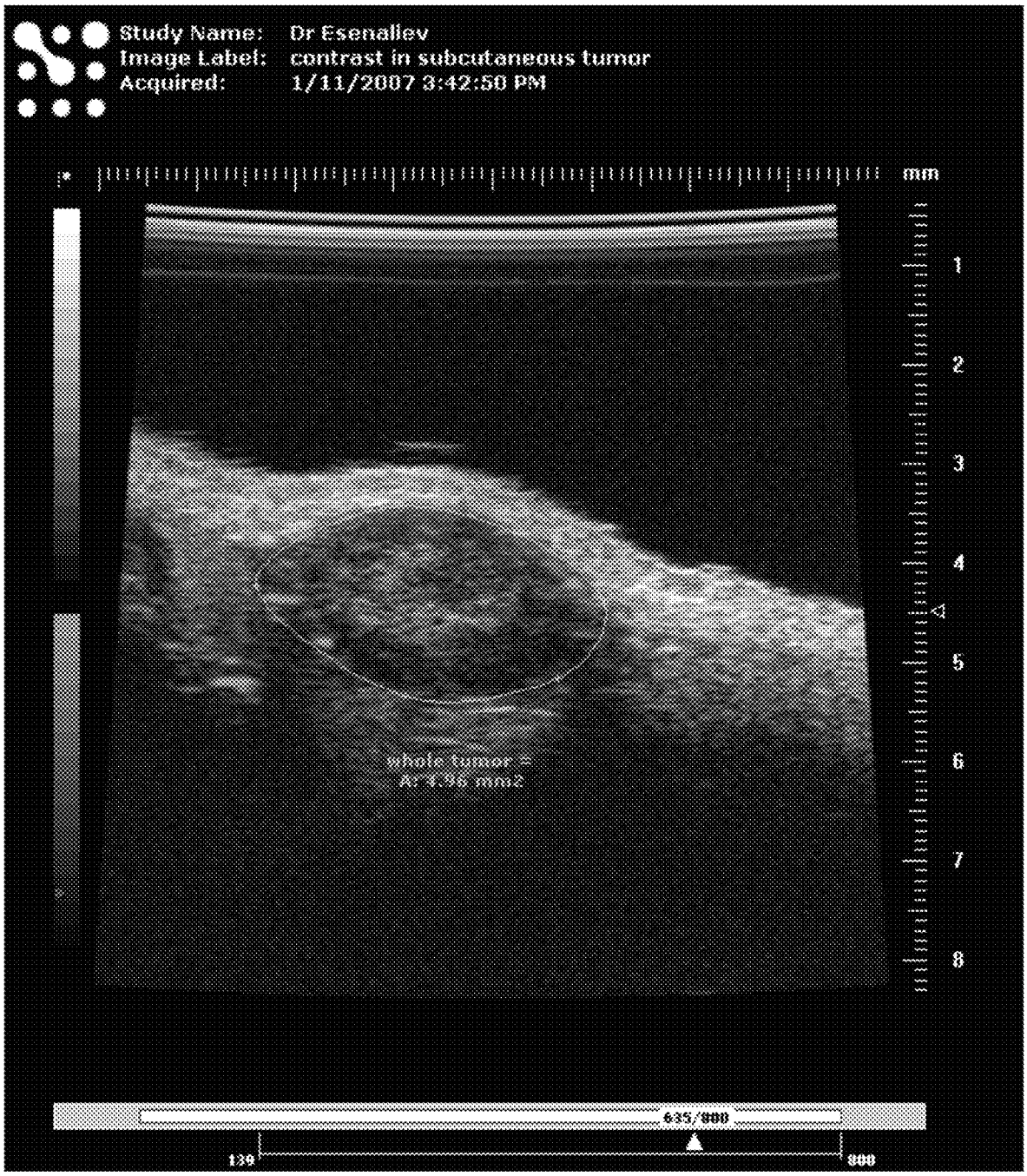
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Image Label: contrast in subcutaneous tumor
Acquired: 1/11/2007 3:42:50 PM
mm
1
2
3
4
5
6
7
8
635/800
139
800

Fig. 9B

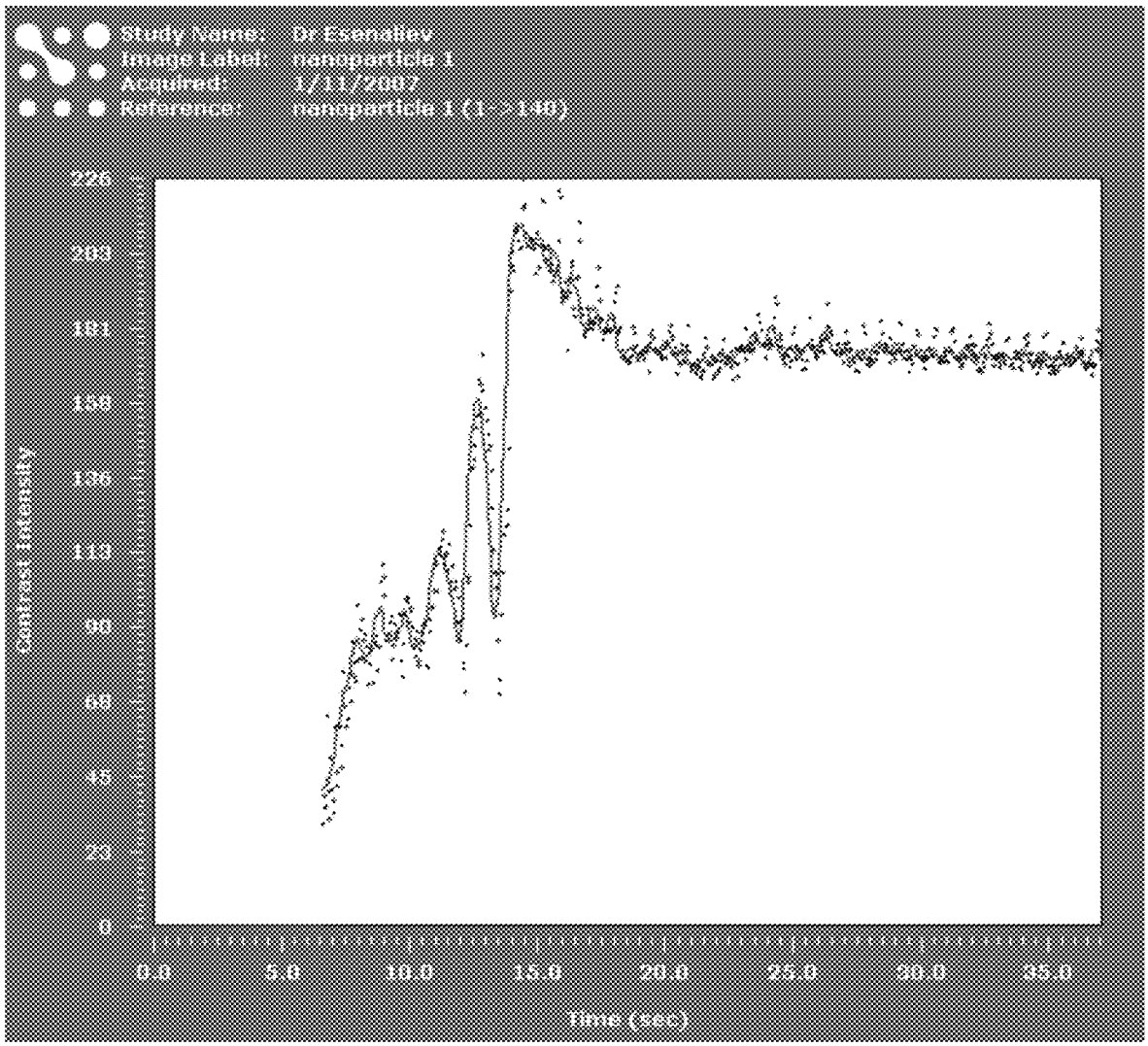

Study Name: Dr Esenaliev
Image Label: nanoparticle 1
Acquired: 1/11/2007
Reference: nanoparticle 1 (1->140)
226
203
181
158
136
113
90
68
45
23
0
Contrast Intensity
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
Time (sec)

Fig. 10A

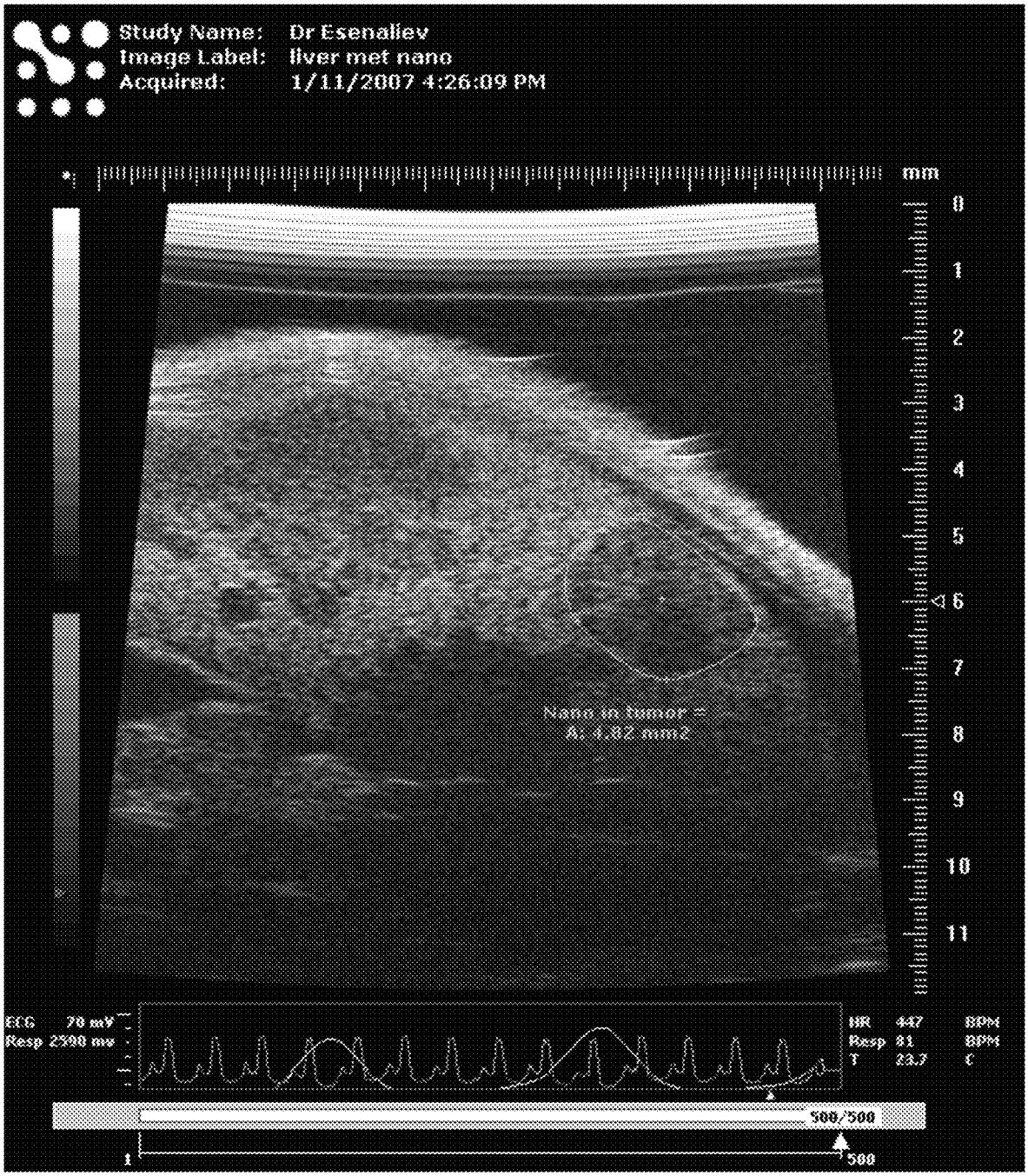

Study Name: Dr Esenaliev
Image Label: liver met nano
Acquired: 1/11/2007 4:26:09 PM
mm
0
1
2
3
4
5
6
7
8
9
10
11
Nano in Tumor =
ECG 70 mV
Resp 2500 mv
HR 447 BPM
Resp 81 BPM
500/500
1
500

Fig. 10B

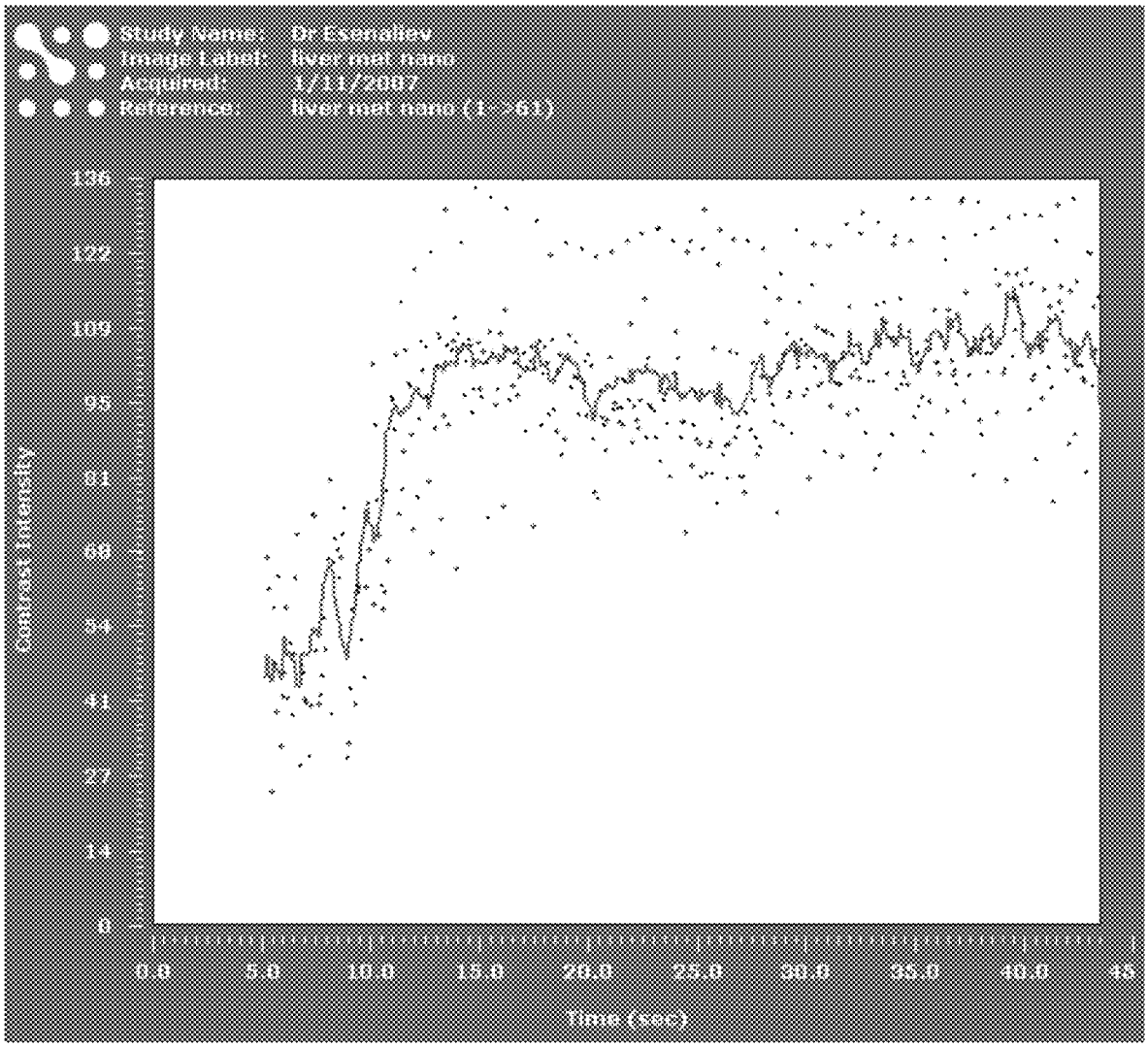

Study Name: Dr Esenaliev
Image Label: liver mat nano
Acquired: 1/11/2007
Reference: liver mat nano (1->61)
Contrast Intensity
136
122
109
95
81
68
54
41
27
14
0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
Time (sec)

Fig. 11A

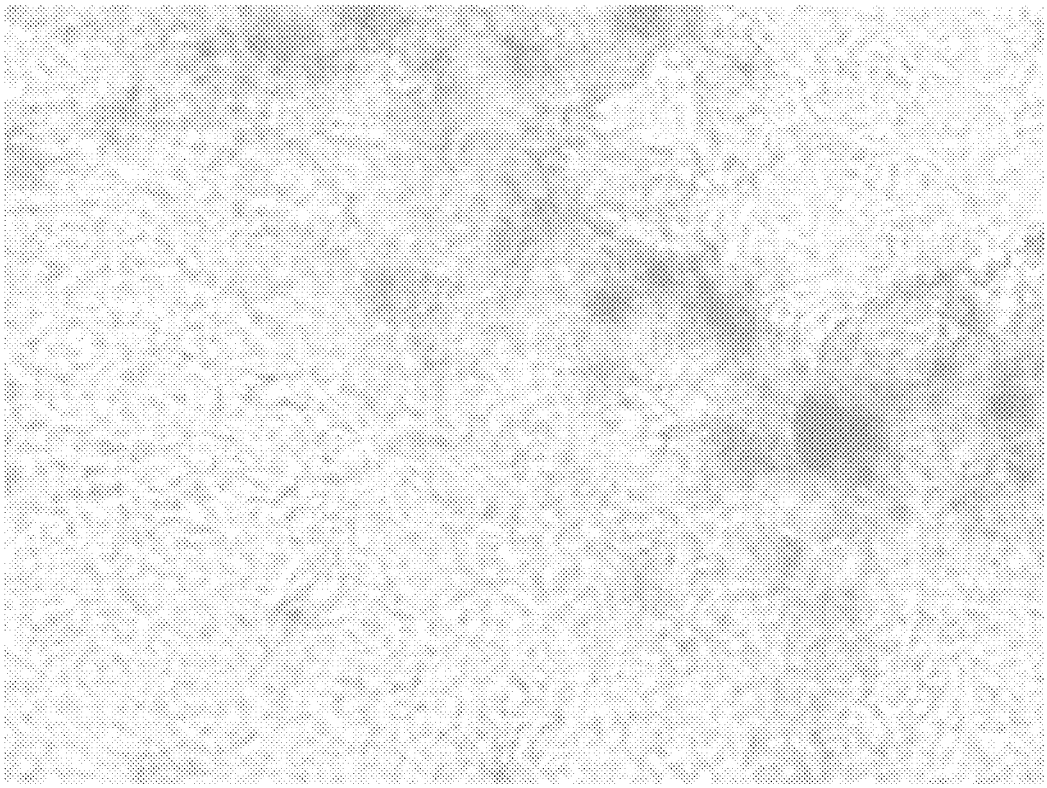


Fig. 11B

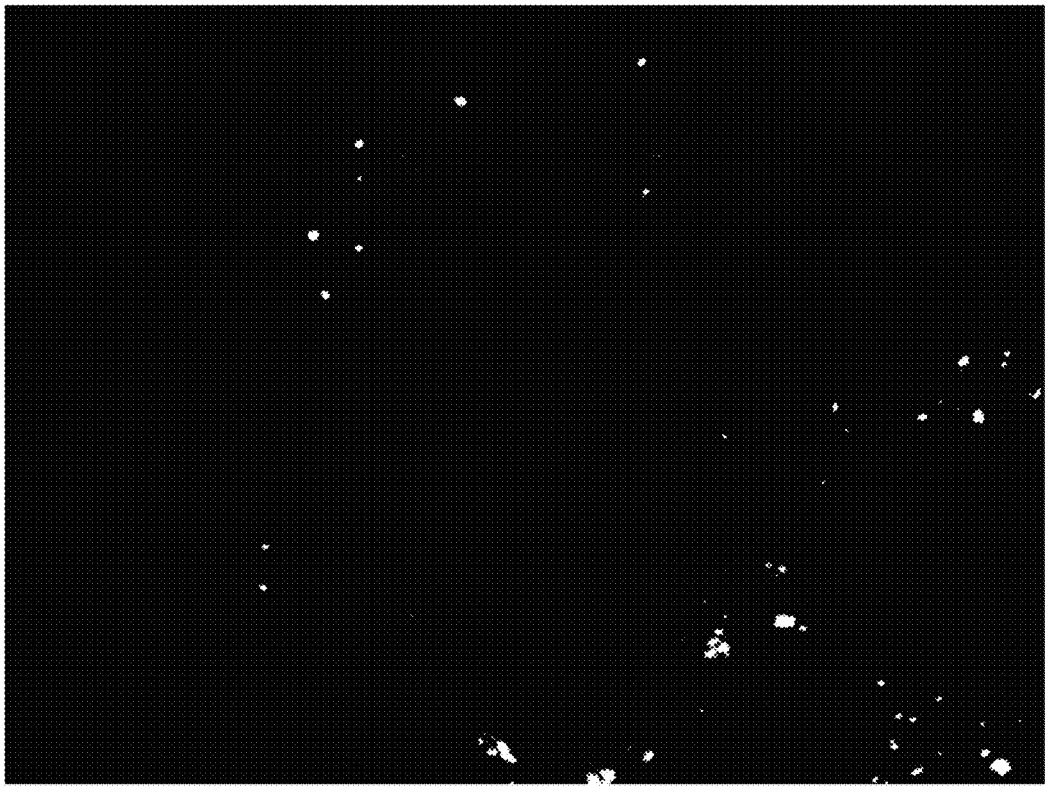


Fig. 12A

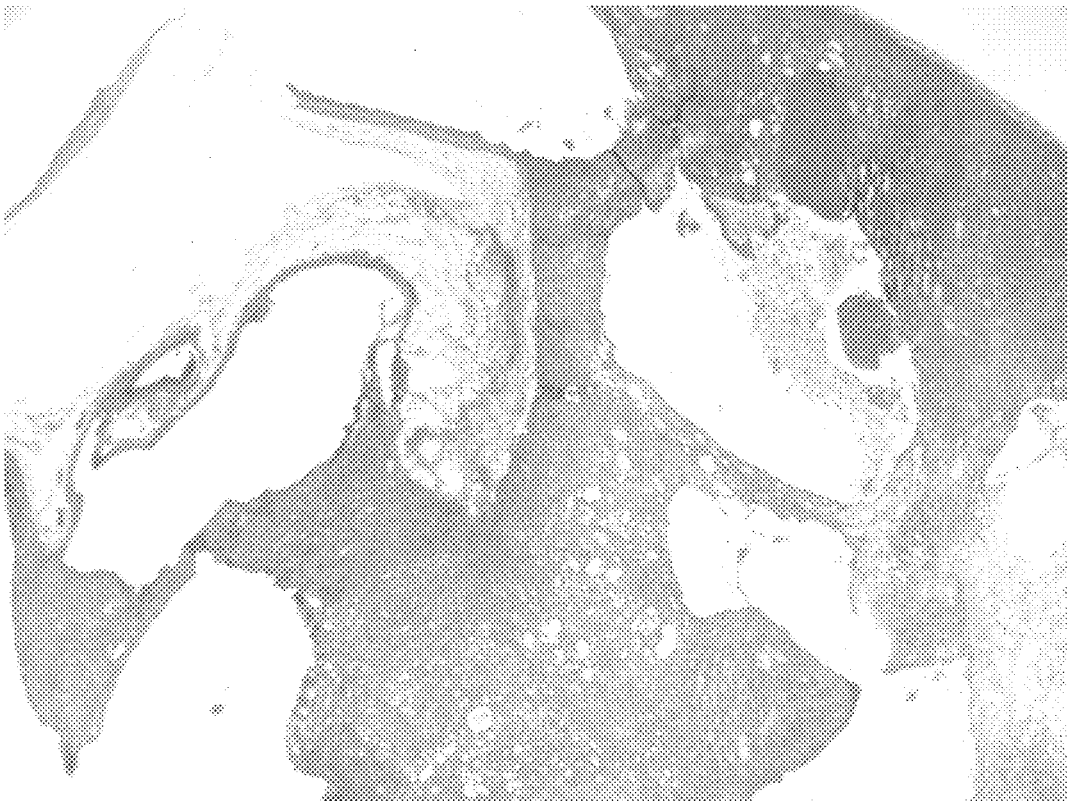


Fig. 12B

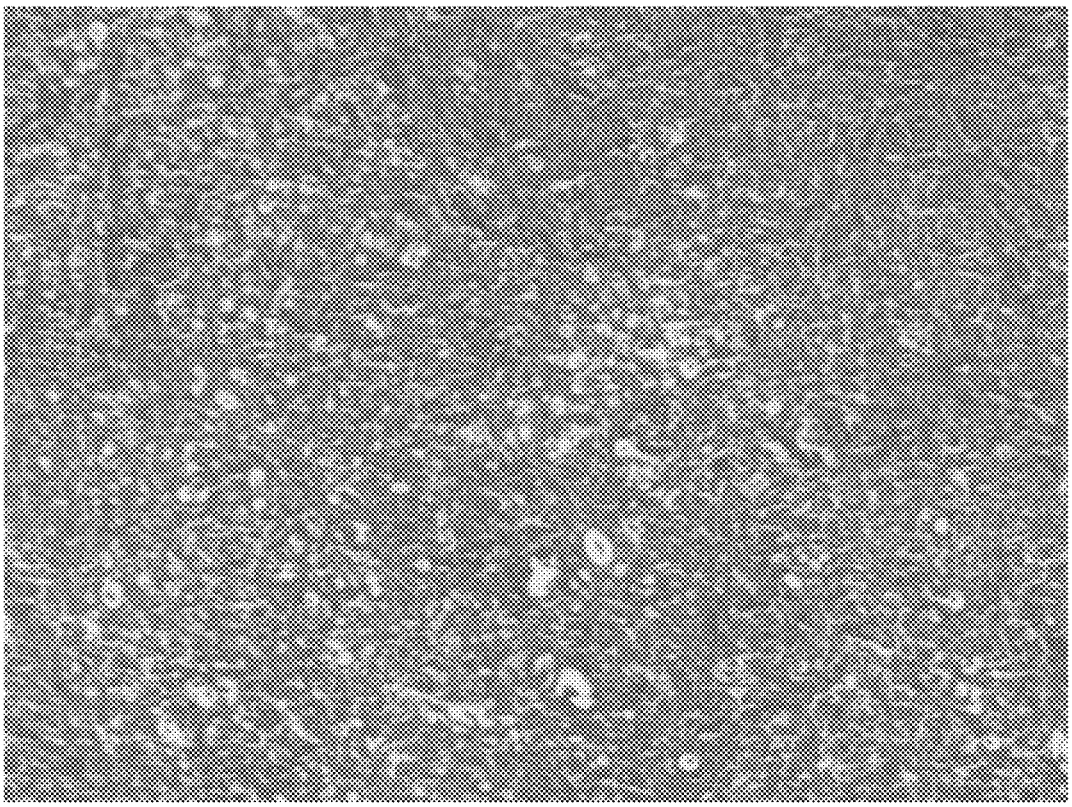


Fig. 13A

FIG. 13B

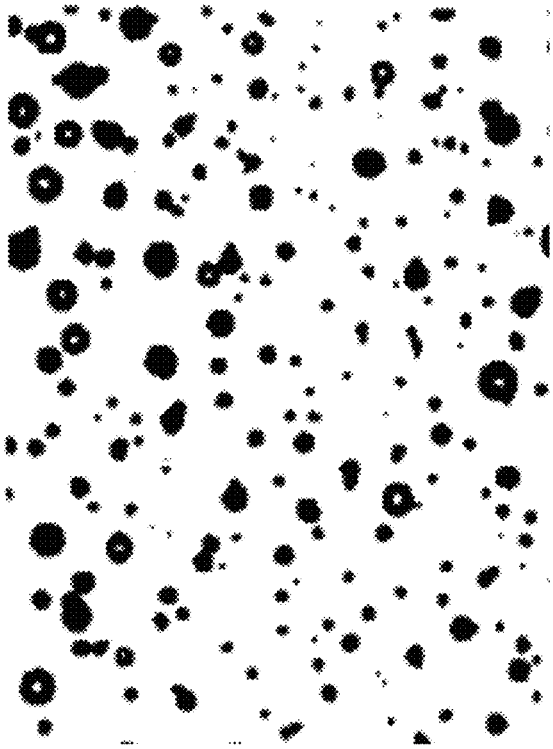


Fig. 13C

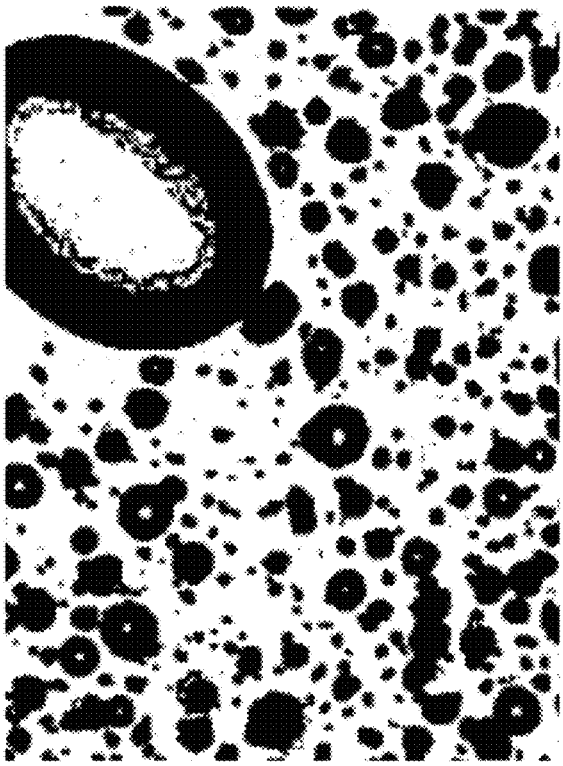


FIG. 13D

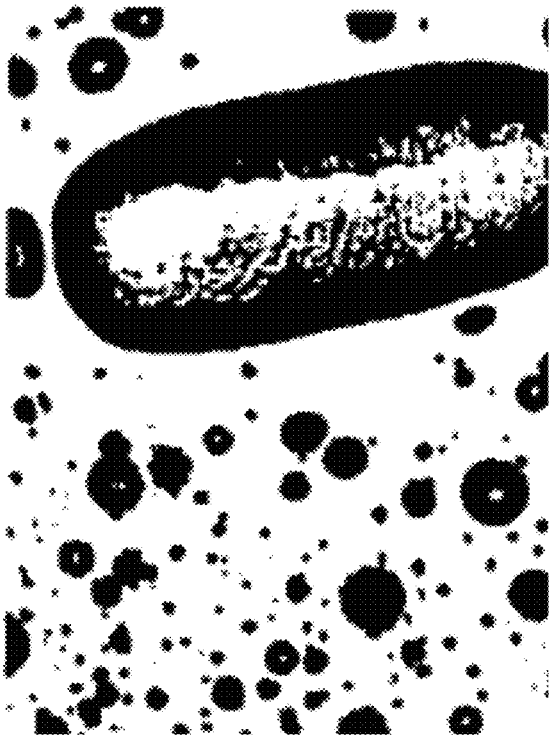


Fig. 14A

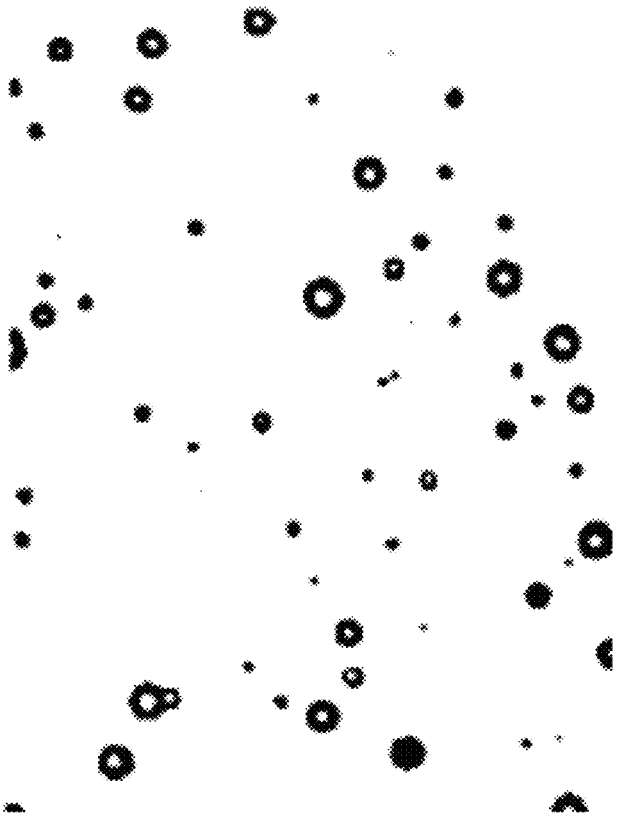


Fig. 14B

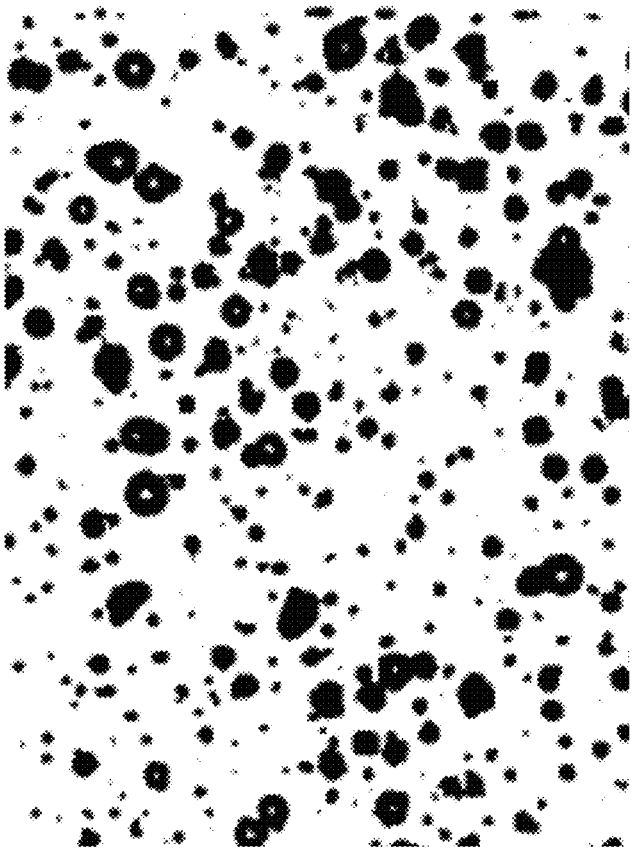


Fig. 15A

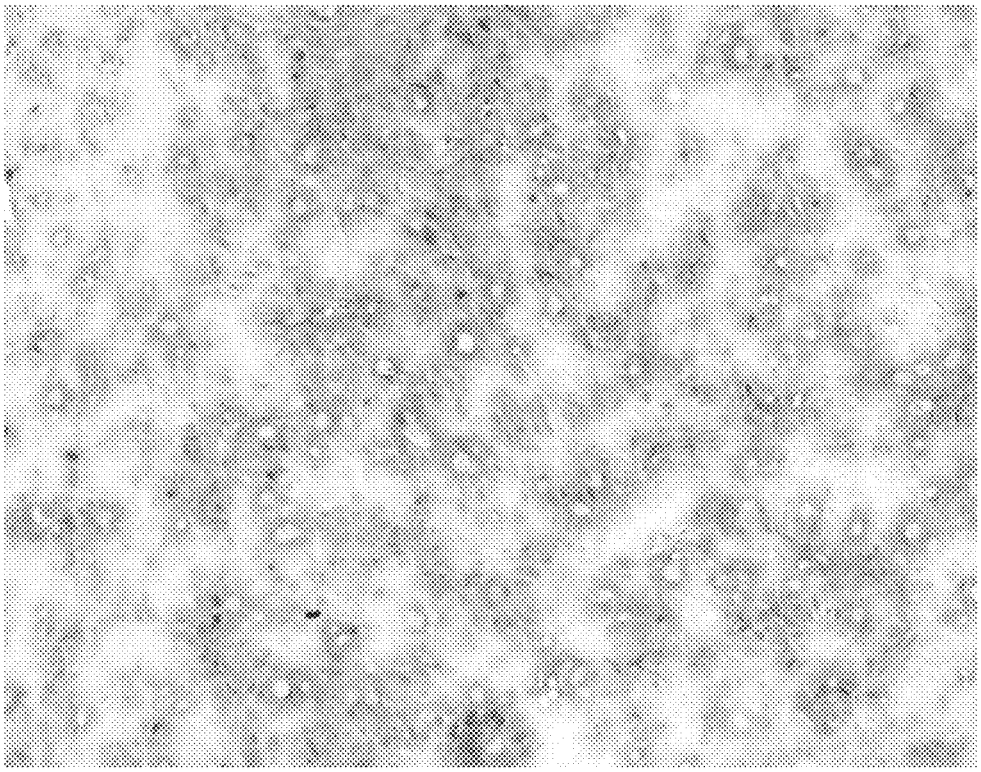


FIG. 15B

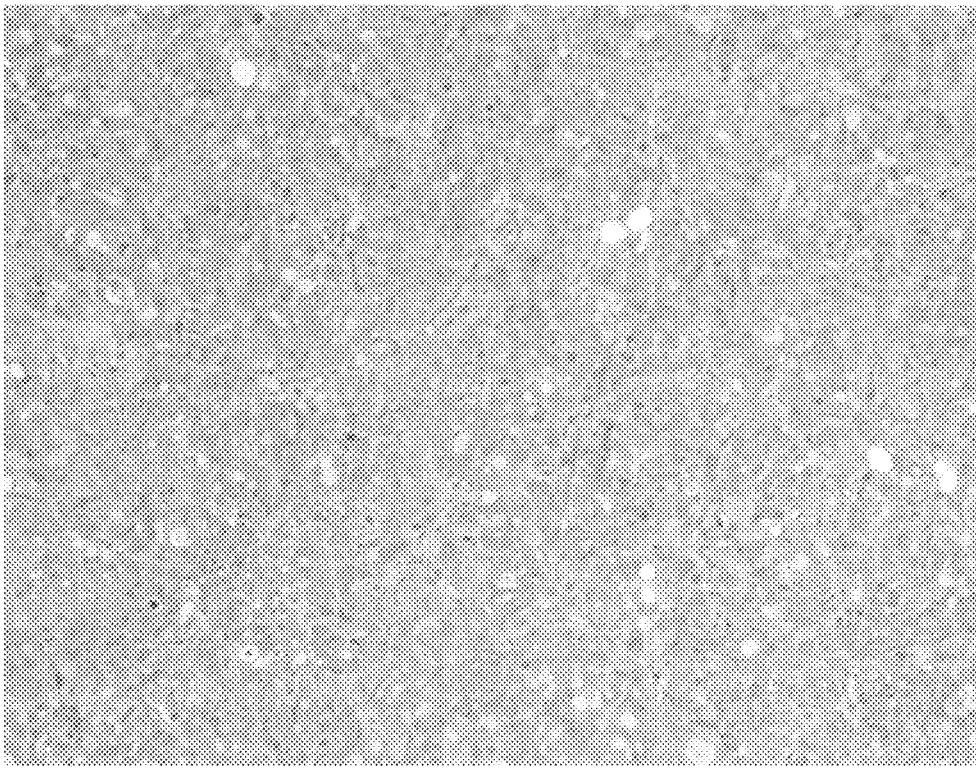


FIG. 16A

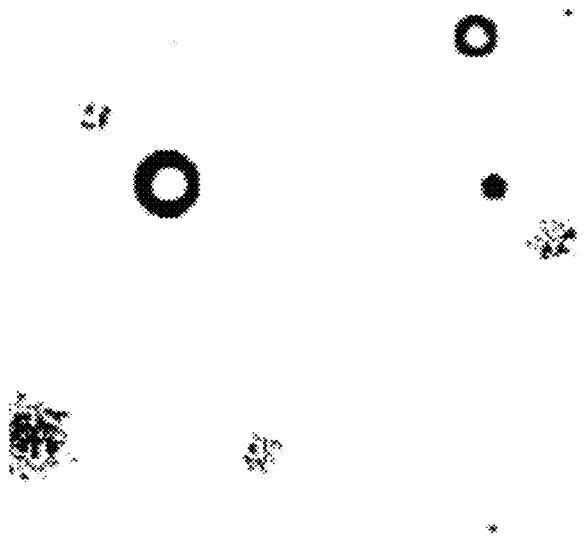
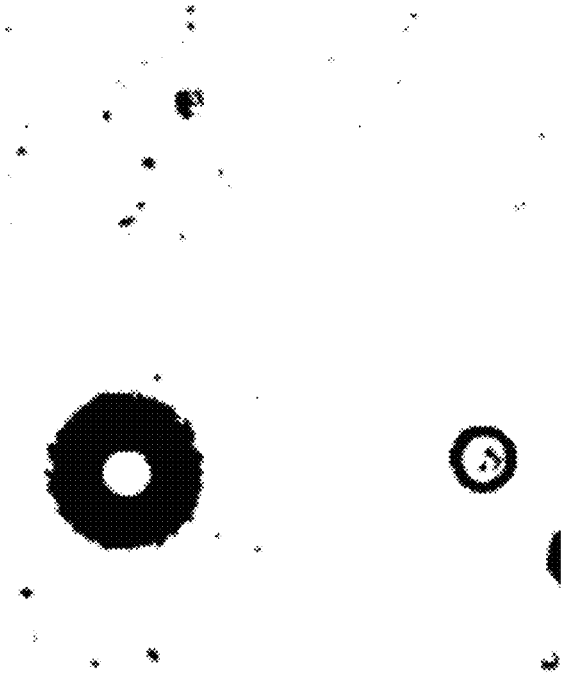


FIG. 16B



NONINVASIVE THERAPIES IN THE TREATMENT OF PATHOGENIC INFECTIONS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of, and claims priority to, U.S. application Ser. No. 15/332,932, filed Oct. 24, 2016, which is a continuation of, and claims priority to, U.S. application Ser. No. 12/821,398, filed Jun. 23, 2010, and issued as U.S. Pat. No. 9,504,824 B2 on Nov. 29, 2016, which in turn claims priority to U.S. Provisional Patent Application Ser. Nos. 61/219,693, filed Jun. 23, 2009, and 61/322,515, filed Apr. 9, 2010, each of which are incorporated herein by reference in their entireties.

BACKGROUND OF THE INVENTION

Field of the Invention

Embodiments of the present invention relate to methods for treating cell components, cells, organelles, organs, and/or tissues with acoustic energy, electromagnetic energy, static or alternating electric fields, and/or static or alternating magnetic fields in the presence or absence of particulate enhancing agents for therapeutic applications including enhancing drug delivery to cell components, cells, organelles, organs, and/or tissues.

More particularly, embodiments of the present invention relate to methods for treating cell components, cells, organelles, organs, and/or tissues with acoustic energy, electromagnetic energy, static and/or alternating electric fields, and/or static and/or alternating magnetic fields in the presence or absence of particulates for therapeutic applications including enhancing drug delivery to cell components, cells, organelles, organs, and/or tissues, where the methods include determining a type of energy and/or field, which produces a desired response in a cell component, a cell type, an organelle, an organ, and/or a tissue. The methods also include irradiating with acoustic and/or electromagnetic energy and/or applying a static and/or alternating electric and/or magnetic fields to the cell component, the cell type, the organelle, the organ, and/or the tissue for a duration, at energy level and/or field level, at a frequency or frequencies sufficient to achieve a desired response in the cell component, the cell type, the organelle, the organ, and/or the tissue. The methods may also include irradiating and/or applying in the presence of particles designed to enhance therapeutic efficacy of the irradiating and/or applying.

Description of the Related Art

U.S. Pat. No. 6,165,440 to Esenaliev disclosed a treatment of solid tumors with acoustic and/or electromagnetic energy in the presence of nanoparticles to effectuate a therapeutic response in the tumor.

U.S. Pat. Nos. 7,144,627, 6,908,496, 6,852,252, 6,778,316, 6,699,724, 6,685,986, 6,685,730, 6,660,381, 6,645,517, 6,530,944, 6,428,811, and 6,344,272 disclose the use of nano-shells to enhance therapeutic treatments in a body through the placement of the nano-shells in the tissue to be treated and the particles thermalize incident radiation causing localized heating of the tissue.

Although acoustic and/or electromagnetic energy in the presence of nanoparticles have been disclosed for therapeutic applications, there is still the need in the art for new or different methods for therapeutic applications capable of being performed in the absence or presence of nanoparticles

SUMMARY OF THE INVENTION

Embodiments of the present invention provide methods for treating cell components, cells, organelles, organs, and/or tissues with acoustic energy, electromagnetic energy, static or alternating electric fields, and/or static or alternating magnetic fields in the presence or absence of particulate enhancing agents for therapeutic applications. For treatments that are performed in the absence of particulate enhancing agents, the frequency, frequencies, frequency spectrum, and amplitude of the acoustic or electromagnetic energy along with mode of radiation—continuous, pulsed, or mixtures thereof—and the field amplitude and mode—continuous, pulsed, variable, or mixture thereof are tuned to the cell components, cells, organelles, organs, and/or tissues being treated. For treatments that are performed in the presence of particulate enhancing agents, the type of agents along with the factors controlling the energy and field properties are tuned to the particulate enhancing agents.

Embodiments of the present invention provide methods for enhancing drug delivery including applying a pharmaceutical agent or agents to an animal including mammals and humans and applying continuous and/or non-continuous acoustic energy, continuous and/or non-continuous electromagnetic energy, static or alternating electric fields, and/or static or alternating magnetic fields to cell components, cells, organelles, organs, and/or tissues to enhance local pharmaceutical agent activity in the components, cells, organelles, organs, and/or tissues.

Embodiments of the present invention provide methods applying continuous and/or non-continuous acoustic energy, continuous and/or non-continuous electromagnetic energy, static or alternating electric fields, and/or static or alternating magnetic fields to cell components, cells, organelles, organs, and/or tissues to damage the components, cells, organelles, organs, and/or tissues and/or alter or modify the properties of the components, cells, organelles, organs, and/or tissues.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention can be better understood with reference to the following detailed description together with the appended illustrative drawings in which like elements are numbered the same:

FIG. **1** depicts a plot relative heating (temperature rise per watt) over the frequency range between about 2 MHZ and about 500 MHZ for various nanoparticles.

FIG. **2** depicts a plot relative heating (temperature rise per watt) over the frequency range between about 2 MHZ and about 500 MHZ for various nanoparticles.

FIG. **3**A depicts scanning Electron Microscopy of PLGA nanoparticles. FIG. **3**B depicts typical size distributions of filtered PLGA nanoparticles (1 mg/mL in water).

FIG. **4** depicts an experimental setup for ultrasound irradiation of tumors in mice and detection of cavitation.

FIG. **5**A depicts a typical cavitation signal obtained from pure water. FIG. **5**B depicts a typical cavitation signal obtained from water with PLGA nanoparticles.

FIG. **6**A depicts a cavitation activity measured in pure water at different ultrasound pressure. FIG. **6**B depicts a cavitation activity measured in water with PLGA nanoparticles at different ultrasound pressure.

FIG. **7**A depicts a cavitation activity measured in vivo in KM20 tumor of a nude mouse after injection of Optison.

FIG. 7B depicts a cavitation activity measured in vivo in KM20 tumor of a nude mouse after injection of PLGA nanoparticles.

FIG. 8A depicts a fluorescence microscopy of tumor irradiated with 1 MHZ ultrasound. FIG. 8B depicts a fluorescence microscopy of non-irradiated tumor.

FIG. 9A depicts a tumor image obtained with the high-resolution ultrasound system. FIG. 9B depicts a kinetics of the PLGA nanoparticles in the tumor shown in FIG. 9A.

FIG. 10A depicts a metastatic cancer in liver (the multiple tumors) obtained with high-resolution ultrasound imaging system. FIG. 10B depicts a penetration of the specially developed biodegradable PLGA nanoparticles in the specific tumor marked in FIG. 10A.

FIG. 11A depicts gene delivery and cell transfection in vivo obtained using interaction of PLGA nanoparticles with pulsed ultrasound. FIG. 11B depicts penetration of biodegradable PLGA nanoparticles in the abnormal tissue after ultrasound irradiation.

FIG. 12A depicts a multiple lesions induced in abnormal tissue using interaction of PLGA nanoparticles with pulsed ultrasound. FIG. 12B shows no damage is induced by the PLGA nanoparticles in non-irradiated (control) part of the abnormal tissue.

FIG. 13A through FIG. 13D depict different extent and severity of damage induced by pulsed electromagnetic heating of absorbing nanoparticles. The fluence of the near infra-red laser pulses was 0.35 J/cm^2, 0.7 J/cm^2, 1.05 J/cm^2, and 1.4 J/cm^2 for FIG. 13A, FIG. 13B, FIG. 13C, and FIG. 13D, respectively.

FIG. 14A and FIG. 14B depict different extent and severity of damage induced by pulsed electromagnetic heating of absorbing nanoparticles. One pulse was used for the sample in FIG. 14A and 10 pulses were used for the sample in FIG. 14B.

FIG. 15A depicts severe damage to abnormal tissue induced by pulsed ultrasound. FIG. 15B depicts no damage in non-irradiated (control) part of the abnormal tissue.

FIG. 16A and FIG. 16B depict damage induced in heterogeneous tissue phantom by pulsed electromagnetic radiation (near infra-red laser pulses). Smaller damaged areas were obtained after irradiation with one pulse at fluence of 1.05 J/cm^2 as shown in FIG. 16A compared to larger damaged areas at fluence of 1.4 J/cm^2 as shown in FIG. 16B.

DETAILED DESCRIPTION OF THE INVENTION

The inventors have found that therapeutically effective treatments can be implemented, where acoustic energy, electromagnetic energy, electric fields, and/or magnetic fields in the absence or presence of particulate enhancing agents are used to induce a therapeutic response in cell components, cells, organelles, organs, and/or tissues. The therapeutic response may be to damage cell components, kill cells, and/or to augment or alter or modify cell components, cells, organelles, organs and/or tissues depending on the therapy need and intended. The energies and/or fields may also enhance drug delivery in the absence of particulate enhancing agents.

Interaction of acoustic and/or electromagnetic energy and/or electric and/or magnetic fields with cell components, cells, organelles, organs and/or tissues can result in heating, sonic vibration, cavitation, electronic excitation, and/or augmentation of other biological, chemical and/or physical properties of the cell components, cells, organelles, organs and/or tissues.

Interaction of acoustic and/or electromagnetic energy and/or electric and/or magnetic fields with pharmaceutical agent or agents being delivery to cell components, cells, organelles, organs and/or tissues can result in enhancing delivery through the heating, sonic vibration, cavitation, electronic excitation, and/or augmentation of other biological, chemical and/or physical properties of the cell components, cells, organelles, organs and/or tissues.

Interaction of radiation with particulate enhancing agents including nanoparticles and/or microparticles may be used for a variety of therapeutic applications. Radiation including, but not limited to, radio-frequency radiation, microwave, low-frequency electromagnetic wave, static electrical fields, magnetic fields, terahertz radiation, infra-red radiation, visible radiation, ultraviolet radiation, as well as ultrasound may be used in therapeutic application. In certain embodiments, a combination radiations may be used for more efficient and safe therapies. In other embodiments, one frequency, a plurality of frequencies or a wide spectrum of frequencies may be used for more efficient and safe therapy. In certain embodiments, the electromagnetic waves (field) are in the frequency range from 0 to about 3×10^{19} Hz. In certain embodiments, the radiation is radio-frequency radiation, microwave radiation, or near infra-red range radiation to provide deep penetration in tissues and optimal interaction with nanoparticles or microparticles. In other embodiments, the radiation may be pulsed, continuous, and/or modulated. In other embodiments, the radiation is pulsed with a pulse duration having a value between about one femtosecond and one second. In other embodiments, ultrasound radiation is using having a frequency between about 20 kHz and about 1 Gigahertz.

Nanoparticles and/or microparticles suitable for use in this invention include, without limitation, metal particles, semiconductor particles, dielectric particles, metal-coated dielectric particles, metal coated semiconductor particles, polymer particles, metal coated polymer particles, bio-compatible polymer particles, bio-degradable polymer particles, or mixtures and combinations thereof. Nanoparticles and/or microparticles may be solid, liquid, gas particles, or mixtures or combinations thereof. It should be recognized that liquid nanoparticles and/or microparticles would be in the form of nano-drops or droplets and/or micro-drops or droplets. It should be recognized that gaseous nanoparticles and/or microparticles would be in the form of nano-bubbles and/or micro-bubbles. In certain embodiments, the nanoparticles may be made of gold, silver, platinum, carbon, graphite, or mixtures and combinations thereof. In other embodiments, the nanoparticles may be nano-shelled particles having a core of one material and a shell of another material. The nanoparticles include, but not limited to, spheres, rods, cylinders, disks, shells, tubes (including single-walled nanotubes), rings, irregular-shaped particles or mixtures and combinations thereof. The size of nanoparticles and microparticles may be between about 1 nanometer and about 100 microns. The nanoparticles and/or microparticles may be injected into blood, interstitially, applied topically, applied locally, subcutaneously, and/or orally depending on application and particle size, shape, and/or material. The nanoparticles and/or microparticles may be delivered using passive delivery or active delivery with targeting agents

Therapeutic applications include, but not limited to, treatments of normal and/or abnormal tissue, stimulation and/or alteration of normal tissue, and/or cosmetic treatment. Therapy of abnormal tissues includes, but not limited to, treatments of malignant tumors or lesions, treatments of benign tumors or lesions, treatments of atherosclerotic

plaques (fibrous, fatty, or calcified), treatments of blood clots, treatments of blood, treatments of amyloid plagues, treatments of neurofibrillary tangles, treatments of fibrous tissues, treatments of fatty tissue, treatments of calcified tissues, treatments of scar tissues, treatments of bone tissues, treatments of hypoxic tissues, treatments of bacteria, and/or treatments of viruses. Therapeutic applications include, but not limited to cancer therapy, atherosclerosis therapy, heart disease therapy, stroke therapy, thrombolysis, therapy of benign prostatic hyperplasia, Alzheimer's disease therapy, therapy of other neurodegenerative disorders, therapy or diabetes, and/or therapy of infectious diseases. Stimulation and/or alteration of normal tissue may be used to improve and/or treat a variety of conditions and diseases including, but not limited to, stimulation and/or alteration of the immune system, stimulation and/or alteration cancer therapy, stimulation and/or alteration therapy or infectious diseases, stimulation and/or alteration ischemic tissues, and/or stimulation and/or alteration of regeneration of tissue. Cosmetic treatment includes, but not limited to, skin rejuvenation, hair removal, hair growth stimulation, fat destruction or removal.

Suitable biocompatible, biodegradable polymers include, without limitation, polylactides, polyglycolides, polycaprolactones, polyanhydrides, polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), poly(amino acids), poly(methyl vinyl ether), poly (maleic anhydride), chitin, chitosan, and copolymers, terpolymers, or higher poly-monomer polymers thereof or combinations or mixtures thereof.

Typically, the polymers will either be surface erodible polymers such as polyanhydrides or bulk erodible polymers such as polyorthoesters. Poly(l-lactic acid) (PlLA), poly(dl-lactic acid) (PLA), poly(glycolic acid) (PGA), polycaprolactones, copolymers, terpolymer, higher poly-monomer polymers thereof, or combinations or mixtures thereof are preferred biocompatible, biodegradable polymers. The preferred biodegradable copolymers are lactic acid and glycolic acid copolymers sometimes referred to as poly(dl-lactic-co-glycolic acid) (PLG). The co-monomer (lactide:glycolide) ratios of the poly(DL-lactic-co-glycolic acid) are preferably between about 100:0 to about 50:50 lactic acid to glycolic acid. Most preferably, the co-monomer ratios are between about 85:15 and about 50:50 lactic acid to glycolic acid. Blends of PLA with PLG, preferably about 85:15 to about 50:50 PLG to PLA, are also used to prepare polymer materials.

PLA, PlLA, PGA, PLG and combinations or mixtures or blends thereof are among the synthetic polymers approved for human clinical use. They are presently utilized as surgical suture materials and in controlled release devices, as well as in other medical and pharmaceutical applications. They are biocompatible and their degradation products are low molecular weight compounds, such as lactic acid and glycolic acid, which enter into normal metabolic pathways. Furthermore, copolymers of poly(lactic-co-glycolic acid) offer the advantage of a large spectrum of degradation rates from a few days to years by simply varying the copolymer ratio of lactic acid to glycolic acid.

Suitable polymers for use in the present invention include, without limitation, biocompatible polymers, biocompatible polymers that are biodegradable and/or bioerodible, i.e., the polymers eventually decompose in the body. The biodegradation and/or bioerodible can be by cellular degradation (e.g., macrophage degradation or the like), chemical degradation (e.g., enzymatic degradation), hydrolysis (e.g., via bodily fluids such as plasma) or other cellular action and/or the degradation or erosion can be due to degradation agents contained within the composition itself (e.g., embedded enzymes, depolymerization agents or the like). Such polymeric substances include polyesters, polyamides, polypeptides and/or polysaccharides or the like.

To enhance bio-degradation of the polymers used in biological application, the compositions of the present invention can also include the addition of enzymes that can facilitate the biodegradation of the polymers used in the composition. Preferred enzymes or similar reagents are proteases or hydrolases with ester-hydrolyzing capabilities. Such enzymes include, without limitation, proteinase K, bromelaine, pronase E, cellulase, dextranase, elastase, plasmin streptokinase, trypsin, chymotrypsin, papain, chymopapain, collagenase, subtilisn, chlostridopeptidase A, ficin, carboxypeptidase A, pectinase, pectinesterase, an oxidoreductase, an oxidase or the like. The inclusion of an appropriate amount of such a degradation enhancing agent can be used to regulate composition duration

The interaction of the nanoparticles or microparticles with electromagnetic radiation can include heating of the particles and surrounding cells and tissues that, in turn, results in hyperthermia, coagulation, explosive evaporation, plasma formation, acoustic wave formation depending on the duration, frequency, energy, and/or power of electromagnetic radiation and repetition rate of electromagnetic pulses (if pulsed radiation is used). These processes result in destruction or alteration of abnormal tissue, and/or stimulation or alteration of normal tissue. The size of the thermal damage area L is dependent on thermal diffusivity $\chi \sim 1.3\times10^{-3}$ cm^2/s (of the tissue). The area L is controlled by varying a pulse duration of the radiation according to equation (1)

$$L \sim (\chi\tau)^{1/2} \quad (1)$$

where τ is pulse duration. For instance, to induce precise damage to subcellular structures, radiation pulses may have a duration up to about 10 microsecond. To induce precise damage to individual cells, radiation pulses may have a duration of the order of 10 microsecond. To induce precise damage to millimeter-sized areas, radiation pulses may have a duration of the order of 1 second. Thus, the areas of damages are controllable by varying the pulse duration of the treating radiation.

In embodiments using acoustic radiation (e.g., ultrasound radiation) the interactions with nanoparticles or microparticles may produce cavitation, acoustic streaming, and/or radiation force that, in turn, may result in mechanical destruction of the tissue. In certain embodiments, the precise damage to a tissue is determined by acoustic frequency, duration, energy, power, and/and pulse repetition rate.

The interaction of the nanoparticles or microparticles with radiation may be used to deliver therapeutic agents. It also may be applied in combination with other therapeutic modalities to achieve higher efficacy and safety.

The inventors have performed studies on the interaction of radiation with nanoparticles to demonstrate heating of nanoparticles by electromagnetic radiation. The inventors used electromagnetic radiation in the radio-frequency (RF) range, frequencies between about 2 MHZ and 500 MHZ. The radiation was generated by an RF generator that provided tunable, continuous wave (CW) RF radiation, which was amplified by an RF amplifier with output power of up to 50 W. A quartz cuvette with two electrodes attached to the sides was specially designed to provide irradiation of nano-

particles in water. The RF radiation was delivered to the electrodes by a high-frequency cable. The heating of the following samples was studied: water, water with surfactant, saline, gold nanoparticles (nano-spheres, nano-rods and nano-shells), silver, copper, nickel, cobalt, iron, carbon nano-tubes (single-walled, double-walled, and multi-walled).

FIG. **1** shows the relative heating (temperature rise per watt) for these samples vs. frequency. FIG. **2** shows the same data, but with water heating subtracted. Our data indicate that in certain embodiments, the frequency range between about 100 MHZ and 500 MHZ is optimal for heating the nanoparticles in an aqueous media. Moreover, all nanoparticles produce higher heating compared to that of water or water with surfactant, while silver nanoparticles produce best heating. While silver nanoparticle have low toxicity, one may coat the silver nanoparticles with gold or other non-toxic materials to further reduce toxicity.

Interaction of Radiation with Cell Components, Cells, Organelles, Organs and/or Tissues

Interaction of radiation with specific tissue parts (including but limited to endogenous nano-, micro-, mm-, or cm-sized particles, layers, compartments), cells, cell organelles, pathogens, or toxins can be used for a variety of therapeutic applications. Radiation includes, but not limited to, radiofrequency, microwave, low-frequency electromagnetic wave, static electrical or magnetic field, terahertz, infra-red, visible, ultraviolet, as well as ultrasound or mixtures and combinations thereof. In certain embodiments, mixtures and combinations of these radiation types may be used for more efficient and safe therapies. In other embodiments, one frequency, a plurality of frequencies, or a wide spectrum of frequencies may be used for more efficient and safe therapies. The electromagnetic waves (field) are in the frequency range between about 0 and about 3×10^{19} Hz. In certain embodiments, the radiation is radiation in the radiofrequency, microwave, or near infra-red range to provide deep penetration in tissues and optimal interaction with tissue parts.

Radiation can be pulsed, continuous wave, or modulated. In certain embodiments, the radiation is pulsed with the pulse duration between about one femtosecond and about one second. In certain embodiments using acoustic radiation, the radiation has a frequency between about 20 kHz and about 1 Gigahertz (ultrasonic radiation). In certain embodiments, the size of tissue parts or cell organelles may be between about 1 nanometer and about 10 centimeters. Tissues include, but not limited to, normal and/or abnormal tissues. Pathogens include, but not limited, to viral pathogens, bacterial pathogens, fungal pathogens, prionic pathogens, or eukaryotic pathogen organisms, or mixtures and combinations thereof.

Therapeutic applications include, but not limited, to therapy of abnormal tissue, stimulation or alteration of normal tissue, cosmetic treatment, inactivation of pathogens or toxins. Therapy of abnormal tissue includes, but not limited, to malignant tumors or lesions, benign tumors or lesions, atherosclerotic plaques (fibrous, fatty, or calcified), blood clots, blood, amyloid plagues, neurofibrillary tangles, fibrous tissues, fatty tissue, calcified tissues, scar tissues, bone tissues, hypoxic tissues, infected tissues. Therapeutic applications include, but not limited to cancer therapy, atherosclerosis therapy, heart disease therapy, stroke therapy, thrombolysis, therapy of benign prostatic hyperplasia, Alzheimer's disease therapy, therapy of other neurodegenerative disorders, therapy of diabetes, therapy of infectious diseases.

Stimulation and/or alteration of normal tissue may be used to improve or treat a variety of conditions and diseases including, but not limited, to stimulation of immune system, cancer therapy, therapy of infectious diseases, ischemic tissues, regeneration of tissue. Cosmetic treatment includes, but not limited, to skin rejuvenation, hair removal, hair growth stimulation, fat destruction or removal.

The interaction of the tissue parts, cells, cell organelles, pathogens, or toxins with electromagnetic radiation can include heating of the tissue parts, cells, cell organelles, pathogens, or toxins and surrounding cells and tissues that, in turn, results in hyperthermia, coagulation, explosive evaporation, electrostriction effects, piezoelectric effects, mechanical stress, acoustic wave formation, plasma formation, depending on duration, frequency, energy, power of electromagnetic radiation and repetition rate of electromagnetic pulses. These processes result in destruction or alteration of abnormal tissue, stimulation or alteration of normal tissue. As stated previously, the size of the thermal damage area L depends on thermal diffusivity $\chi\sim1.3\times10^{-3}$ cm^2/s (of the tissue). The area L is controlled by varying the pulse duration of the radiation according to equation (1)

$$L\sim(\chi\tau)^{1/2} \quad (1)$$

where τ is pulse duration. For instance, to induce precise damage to subcellular structures one can use short pulses with duration up to about 10 microsecond, to induce precise damage to individual cells one can use pulses with duration of the order of 10 microsecond, to induce precise damage to millimeter-sized areas one can use pulses with duration of the order of 1 second, etc.

Interaction of ultrasound with the tissue parts, cells, cell organelles, pathogens, or toxins may produce cavitation, acoustic streaming, and radiation force that, in turn, result in mechanical destruction of the tissue parts, cells, cell organelles, pathogens, or toxins. One can produce precise damage to tissues by varying ultrasound frequency, duration, energy, power, and pulse repetition rate.

Selective damage to the tissue parts, cells, cell organelles, pathogens, or toxins may be produced by the radiation when contrast in electromagnetic (radiofrequency, microwave, low-frequency electromagnetic wave, static electrical or magnetic field, terahertz, infra-red, visible, ultraviolet) or acoustic properties of the tissue parts, cells, cell organelles, pathogens, or toxins is substantial.

Optimization of radiation parameters (duration, frequency, energy, power of electromagnetic radiation and repetition rate of electromagnetic pulses and ultrasound frequency, duration, energy, power, and pulse repetition rate) may be used to improve selective damage to the tissue parts, cells, cell organelles, pathogens, or toxins and to improve therapeutic outcome.

The interaction of radiation with the tissue parts, cells, cell organelles, pathogens, or toxins may be used for delivery of therapeutic agents. The radiation may also be applied in combination with other therapeutic modalities for higher efficacy and safety. This noninvasive therapy may also be used for therapy of human patients as well as animals including companion animals.

One can use exogenous dyes, electromagnetic radiation active, or ultrasound radiation active substances to enhance therapeutic effects. Nanoparticles and microparticles may be used to enhance the therapeutic effect of radiation as described above.

Proteins, lipids, nucleic acids, carbohydrates, water and other molecules, organelles, tissue parts, pathogens have higher absorption at some wavelengths and frequencies

compared to the other constituents. This allows for selective damage to these molecules, organelles, tissue parts, in particular, if pulsed radiation is used. For instance: 1) higher absorption by cancer cell nucleus results in selective damage to cancer cells that may be used for cancer therapy; 2) higher absorption by peptidoglycan and/or lipids results in selective damage to bacterial membrane that may be used for therapy of infectious diseases and inactivation of bacteria in the body or in surrounding environment including, but limited to, water, food, drugs, or during organ or tissue transplantations; 3) higher absorption by nucleic acids or proteins results in selective damage to viral pathogens that may be used for therapy of infectious diseases and inactivation of bacteria in the body or in surrounding environment including, but limited to, water, food, drugs, or during organ or tissue transplantations; 4) higher absorption by proteins results in selective damage to amyloid plaques that may be used for therapy of neurodegenerative disorders. Moreover, one may use difference in size between normal and abnormal cells or cell organelles for selective damage to abnormal cells, organelles, and tissues to provide better therapeutic outcome. For instance, cancer cell nucleus is typically larger than that of normal cell. This can be used for safe and efficient cancer therapy because heat diffusion from cancer cell nucleus is slower than from the nucleus of the normal cell.

These examples are given for the purpose of demonstration of potential therapies, but not for limitations of this technology.

The present technology may be used in combination with other therapeutic modalities including, but not limited, to drug therapy (such as chemotherapy), biotherapy, surgery, conventional radiation therapy, physiotherapy. In certain embodiments, wearable acoustic or electromagnetic devices or transducers of acoustic or electromagnetic energy are used to provide long duration of treatment. The acoustic or electromagnetic energy may be used to provide treatment in a hospital, inpatient, outpatient, home environment or during everyday normal activity for more efficient therapy.

The present technology may be used in combination with imaging technologies and procedures to improve therapy or imaging outcome.

These forms of radiation may be delivered using optical fibers, electromagnetic antennas, acoustic transducers in non-contact or contact mode by attaching them to the skin surface, transcutaneously, interstitially, endoscopically, or by using whole body irradiation.

Ultrasonic Treatments to Enhance Drug Delivery

Biodegradable PLGA Nanoparticles

The PLGA nanoparticles have the following advantages: they are biodegradable, they can be delivered in tumor blood vessels at higher concentrations compared to microparticles or gas micro-bubbles due to the EPR effect, they provide cavitation for longer time, and they provide cavitation at low ultrasound pressure. PLGA is a biodegradable polymer which is being used in patients as a material for surgical sutures. Our laboratory manufactures them with double (water/oil)/water (W/O)/W emulsion solvent evaporation technique using biodegradable polymer Poly(D,L-lactide-co-glycolic acid **50**:**50**, PLGA).

We used microscopy techniques (SEM and optical) as well as particle sizers to evaluate PLGA particle size and structure. FIG. **3**A shows a SEM picture of PLGA nanoparticles. FIG. **3**B shows a typical particle size distribution of filtered PLGA nanoparticles (about 200 nm) used in the studies.

Referring to FIG. **4**, an embodiment of an ultrasound system of this invention, generally **400**, is shown to include an irradiation focusing transducer **402** and a receiving focusing transducer **404**. The irradiation focusing transducer **402** connected to a power amplifier **406**, which is connected to a signal generator **408**. The irradiation focusing transducer **402** has a resonance frequency of 1 MHZ and the receiving focusing transducer **404** has a resonance frequency of 5 MHZ, which is adapted to detect cavitation signals and activity at 5 MHZ. The signal generator **408** is a pulse/function generator (8116A, Hewlett-Packard) and generates an ultrasound signal **410**. The power amplifier **406** is an Rf Power amplifier (2100L ENI) and amplifies the ultrasonic signal **410** to produce an amplified ultrasonic signal **412**. The generator **408** and the amplifier **406** were used for generation and amplification of electrical signals for the irradiating the irradiation transducer **402**. The system **400** allows measuring cavitation signals with minimal noise associated with other non-linear acoustic effects. In certain embodiments, the system **400** irradiates with short ultrasound pulses having a duration between about 100 ns to about 1 ms with a repetition rate between about 1 Hz to about 1 KHz to induce cavitation. In other embodiments, the system **400** irradiates with short ultrasound pulses having a duration of about 30 μs with a repetition rate of about 20 Hz to induce cavitation. The irradiation focusing transducer **402** is positioned to focus ultrasonic radiation **414** on a tumor **416**, with the receiving focusing transducer **404** positioned to receive an output signal **418** from the irradiated tumor **416**. The transducer **404** converts the output signal **418** into an electronic signal **420**. The signal **420** from the transducer **404** is passed through a high pass filter **422** resulting in a filter signal **424**, which is then forwarded to a signal amplifier **426** resulting in an amplified, filter signal **428**. The amplified signal **428** from the signal amplifier **426** and an output **430** from the signal generator **408** are passed through an ADC board **432** to form an ADC output signal **434**. The output signal **428** of the ADC board **426** is forwarded to a computer **436** via a bi-directional communication GPIB cable **438**.

We studied cavitation threshold and activity in pure water and in water with PLGA nanoparticles as well as in vivo in nude mice with tumors. FIG. **5**A and FIG. **5**B show cavitation signals obtained at same ultrasound pressure from pure water and from water with PLGA nanoparticles, respectively. The cavitation signal obtained from water with PLGA nanoparticles was significantly greater. We integrated the signals by using a procedure described in detail in I. V., Evers B. M., Ashitkov T. V., Bartels C., Larin K. V., Esenaliev R. O. Enhancement of Drug Delivery in Tumors by Using Interaction of Nanoparticles with Ultrasound Radiation. Technology in Cancer Research and Treatment, v. 4(2), 2005, pp. 217-226, to measure cavitation activity at different pressure (cavitation activity is proportional to concentration of cavitation bubbles and strength of individual cavitation events). FIG. **6**A and FIG. **6**B show cavitation activity measured in pure water and in water with PLGA nanoparticles, respectively. Ultrasound pressure (right Y-axis) was increased step by step during the measurements. The cavitation activity was substantially greater in water with PLGA nanoparticles than that measured in pure water. These experiments allowed to measure cavitation threshold (sharp increase of cavitation activity) which was 27 bar for water with PLGA nanoparticles at these experimental conditions. No sharp increase of cavitation activity was detected in pure water because cavitation threshold was very high.

Our in vivo experiments were performed with mice bearing KM20 tumors. We injected Optison or PLGA nanoparticles in the tail vein and measured cavitation signals and activity using same approach as in the experiments with water. FIG. **7**A shows cavitation activity measured from a tumor of a mouse injected with Optison prior to irradiation. We detected cavitation activity with the threshold of about 48 bar. However, the cavitation activity decreased rapidly due to degradation of Optison upon ultrasound irradiation. Injection of another mouse with PLGA nanoparticles (24 hours prior to irradiation to allow for accumulation of the nanoparticles in tumors) also induced cavitation activity in the irradiated tumor (with almost same threshold) as shown in FIG. **7**B. However, PLGA nanoparticles produce stable cavitation for a longer time because they degrade at a much slower rate. These studies demonstrate that PLGA nanoparticles: (1) substantially lower cavitation threshold; (2) produce cavitation in vivo; and (3) produce more stable cavitation in vivo compared with that obtained with Optison.

Model Macromolecular Drug

Rhodamine-dextran (MW=2,000 kDa) (Sigma, Co.) was used as a model drug with high molecular weight. It was injected in the tail vein prior to irradiation (0.1 mL, 1% solution in saline). Tumor blood vessels were stained by using CD-31. We performed a pilot study with 1-MHZ ultrasound in vivo and the rhodamine-dextran and Optison. FIG. **8**A shows much deeper penetration of rhodamine-dextran in KM20 tumor irradiated by 1-MHZ ultrasound compared to non-irradiated tumor as shown in FIG. **8**B.

We also monitored kinetics of the PLGA nanoparticles in tumors by using a high-resolution ultrasound imaging system. The system has the ability to visualize and quantify tumors, hemodynamics, and therapeutic interventions with resolution down to 30 microns noninvasively and in real time. FIG. **9**A shows an image of a DU 145 prostate tumor in a nude mouse obtained with the system. Injection of the PLGA nanoparticles in the mouse demonstrated almost constant concentration of the PLGA nanoparticles 15 seconds after the injection as shown in FIG. **9**B. This effect resulted from competition of two processes: 1) the decrease of nanoparticle concentration in blood and 2) the increase of their concentration in the tumor blood vessels due to the EPR effect. These data indicate that the system is capable of detecting the nanoparticles in tumors in vivo and that these nanoparticles are have very strong interaction with ultrasound and can be used for efficient drug delivery.

Noninvasive Therapy Applications

All these methods and systems may be used without limitations in humans (both in patients and in healthy individuals) and in any animals including mammals and companion animals (such as dogs, cats, etc.).

All these methods and systems may be used without limitations in tissues and body fluids (blood, etc.), outside of animal bodies including mammal bodies and human bodies. These methods and systems may be used for therapy of abnormal tissues or stimulation of normal tissue during tissue or organ transplantation, blood transfusion, hemodialysis, bypass surgery, and other therapies of tissues and body fluids outside of animal bodies including mammal bodies and human bodies.

Therapies with Nanoparticles and Radiation

We developed biodegradable PLGA nanoparticles for drug delivery in abnormal tissues and for therapy without drugs. FIG. **10**A shows metastatic cancer in liver (the multiple tumors) obtained with a high-resolution ultrasound imaging system. FIG. **10**B shows penetration of biodegradable PLGA nanoparticles in the specific tumor marked in FIG. **10**A. These data demonstrate that these methods and systems may be used for PLGA nanoparticle-based therapy of metastatic tumors (and other abnormal lesions) in liver and other organs.

We also developed a method to load drugs and genes in the PLGA nanoparticles. FIG. **11**A shows gene delivery and cell transfection in vivo obtained using interaction of the PLGA nanoparticles (loaded with beta-gal) with pulsed ultrasound. FIG. **11**B shows penetration of the biodegradable PLGA nanoparticles in the abnormal tissue after ultrasound irradiation.

FIG. **12**A shows multiple lesions induced in abnormal tissue using interaction of the PLGA nanoparticles with pulsed ultrasound. FIG. **12**B shows no damage is induced by the PLGA nanoparticles in non-irradiated (control) part of the abnormal tissue.

FIG. **13**A-FIG. **13**D shows different extent and severity of damage (microbubbles) in abnormal tissue phantom induced by pulsed electromagnetic heating of absorbing carbon nanoparticles. The fluence of the near infra-red laser pulses was 0.35 J/cm^2, 0.7 J/cm^2, 1.05 J/cm^2, and 1.4 J/cm^2 for FIG. **13**A, FIG. **13**B, FIG. **13**C, and FIG. **13**D, respectively. Each sample was irradiated with 100 pulses.

FIG. **14**A and FIG. **14**B show different extent and severity of damage (microbubbles) in abnormal tissue phantom induced by pulsed electromagnetic heating of absorbing carbon nanoparticles. One pulse was used for the sample in FIG. **14**A and 10 pulses were used for the sample in FIG. **14**B. The fluence of the near infra-red laser pulses was 1.05 J/cm^2 for both samples.

The data in FIG. **13**A-FIG. **13**D and FIG. **14**A and FIG. **14**B demonstrate that, by varying the parameters of electromagnetic radiation, one can control the extent and severity of the damage to abnormal tissue and limit the damage to surrounding normal tissue. For instance, uniform and precise damage may be induced in the abnormal tissue, without damage to normal tissue, when optimal parameters of radiation are used as shown in FIG. **13**B and FIG. **14**B.

Therapies without Nanoparticles (Therapy with Radiation Only)

FIG. **15**A shows severe damage to abnormal tissue in vivo induced by pulsed ultrasound. FIG. **15**B shows no damage in non-irradiated (control) part of the abnormal tissue.

FIG. **16**A and FIG. **16**B show damage induced in heterogeneous tissue phantom by pulsed electromagnetic radiation (near infra-red laser pulses). The areas with higher absorption have damage (microbubbles), while the areas with lower absorption do not have the damage. Smaller damaged areas were obtained after irradiation with one pulse at fluence of 1.05 J/cm^2 as shown in FIG. **16**A compared to larger damaged areas at fluence of 1.4 J/cm^2 as shown in FIG. **16**B. These data demonstrate that one can induce selective damage to abnormal areas of tissues with pulsed electromagnetic heating of the areas. Moreover, by optimizing the electromagnetic wave parameters, one may control the size of the damaged areas to avoid or minimize damage to normal tissues.

All references cited herein are incorporated by reference. Although the invention has been disclosed with reference to its preferred embodiments, from reading this description those of skill in the art may appreciate changes and modification that may be made which do not depart from the scope and spirit of the invention as described above and claimed hereafter.

I claim:

1. A method for treating organs and/or tissues infected with pathogens comprising:

applying radiofrequency energy in a frequency range between about 2 MHz and about 500 MHz to the organs and/or tissues infected with the pathogens, wherein the radiofrequency energy is tuned to limit damage to the organs and/or tissues being treated while maximizing damage to the pathogens according to a set of parameters including one or more of frequency, pulse duration, pulse repetition rate, treatment duration, and amplitude such that the pathogens selectively absorb the radiofrequency energy resulting in destruction of the pathogens with limited damage to the organs and/or tissues.

2. The method of claim **1**, wherein the pathogens are one or more of bacterial pathogens, viral pathogens, fungal pathogens, prionic pathogens, and eukaryotic pathogens.

3. The method of claim **2**, wherein the pathogens are bacterial pathogens and bacterial cell membranes and/or walls of the bacterial pathogens are selectively damaged by the radiofrequency energy applied with a pulsed non-laser radiofrequency source with a pulse duration of up to 10 microseconds.

4. The method of claim **2**, wherein the pathogens are bacterial pathogens and bacterial cell membranes and/or walls of the bacterial pathogens are selectively damaged by the radiofrequency energy applied with a pulsed non-laser radiofrequency source with a pulse duration of up to 10 nanoseconds.

5. The method of claim **1**, further comprising topically applying a pharmaceutical agent or agents to an animal including mammals and humans to enhance local pharmaceutical agent activity in the organs and/or tissues of the animal.

6. A method for treating organs and/or tissues infected with pathogens comprising:

applying near-infrared radiation to the organs and/or tissues infected with the pathogens, wherein the near-infrared radiation is applied without addition of an enhancing agent and wherein the near-infrared radiation is tuned to limit damage to the organs and/or tissues being treated while maximizing damage to the pathogens according to a set of parameters including one or more of wavelength, fluence, pulse duration, pulse repetition rate, treatment duration, and amplitude such that the pathogens absorb the near-infrared radiation resulting in selective destruction of the pathogens with limited damage to the organs and/or tissues.

7. The method of claim **6**, wherein the pathogens are one or more of bacterial pathogens, viral pathogens, fungal pathogens, prionic pathogens, and eukaryotic pathogens.

8. The method of claim **6**, wherein the pathogens are bacterial pathogens and bacterial cell membranes and/or walls of the bacterial pathogens are selectively damaged by the near-infrared radiation applied with a pulsed near-infrared laser at a pulse duration of up to 10 microseconds.

9. The method of claim **6**, wherein the pathogens are bacterial pathogens and bacterial cell membranes and/or walls of the bacterial pathogens are selectively damaged by the near-infrared radiation applied with a pulsed near-infrared laser at a pulse duration of up to 10 nanoseconds.

10. The method of claim **6**, wherein the near-infrared radiation is pulsed at a fluence range between about 0.35 J/cm^2 and 1.4 J/cm^2.

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