MONITORING OF CONVECTION ENHANCED DRUG DELIVERY

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ABSTRACT

A method for determining a response of a tissue to a destructive treatment is disclosed. The method comprises providing magnetic resonance images of the tissue, and using the magnetic resonance images for determining the response of the tissue to the destructive treatment. Also disclosed is a method for monitoring convection during direct convective interstitial infusion using a pharmaceutical composition which comprises a therapeutic agent and an MRI contrast agent.
Fig. 1

10

MRI apparatus 14

image processing unit 18

destructive treatment apparatus 16

tissue 12
begin 20

21 provide baseline MR images

22 initiate treatment

23 provide MR images

24 determine the response of the tissue

end 26

Fig. 2
begin

30

32
provide a pharmaceutical composition

33
provide baseline MR images

34
pressure the pharmaceutical composition through a direct convective interstitial infusion catheter

36
image a region containing the tissue

38
monitor convection

end

Fig. 3
Fig. 4
begin

52
provide images

54
analyze the images to identify regions of different catheter acceptance levels

56
mark the regions in a distinctive manner

end

Fig. 5a

Fig. 5b
Fig. 6
60 start

62 place a direct convective interstitial infusion catheter

64 pressure the composition through the catheter

66 image the tissue by MRI

67 assess convection

68 convection satisfactory?

yes

71 image the tissue by MRI

72 assess non-specific toxicity

74 non-specific toxicity is low?

no

76 reformulate the composition

Fig. 24

80 end

70 increase viscosity no
MONITORING OF CONVECTION ENHANCED DRUG DELIVERY

FIELD AND BACKGROUND OF THE INVENTION

[0001] The present invention relates to tissue and/or drug monitoring, and, more particularly, to a method and system for monitoring convection and/or cytotoxic response of tissue during or subsequently to convective or other destructive treatments of the tissue.

[0002] In the area of drug delivery into the central nervous system (CNS), in particular for the treatment of neurological diseases, there have been extensive efforts for devising methods for delivering therapeutic agents into the desired neurological site. Peripheral administration of therapeutic agents for the treatment of CNS pathologies is mostly inefficient due to poor penetration of most drugs across the blood brain barrier (BBB). Direct drug delivery methods, such as direct injection, intraventricular instillation, intraventricular topical application, chronic low-flow microinflation and controlled release from polymer implants, are restricted by the poor diffusion of drug through the tissue. Therefore, these drug delivery methods are only useful for treating a small volume of tissue surrounding the drug source.

[0003] Generally, diffusion of a compound in a tissue depends on the free concentration gradient and the diffusivity of the compound in the tissue. The diffusion in the tissue is slow for high molecular weight compounds, and higher for low molecular weight compounds. For the latter, however, capillary forces and oftentimes metabolism generally limit the diffusion efficiency and therapeutic drug levels can be obtained only close (a few millimeters) to the source of drug.

[0004] U.S. Pat. No. 5,720,720, the contents of which are hereby incorporated by reference, discloses a drug delivery technique known in the literature as “Convection-Enhanced Drug Delivery” and abbreviated to CED or CEDD. In this technique drugs delivery into the brain tumor is effected by application of pressure gradients (as opposed to concentration gradient). Specifically, CED involves positioning the tip of an infusion catheter within the brain tissue and supplying the drug through the catheter while maintaining a positive pressure gradient from the tip of the catheter during infusion. The catheter is connected to a pump which delivers the drug and maintains the desired pressure gradient throughout delivery of the drug. Drug delivery rates are typically about 0.5 to about 4.0 ml/min with infusion distances of order of centimeters. This method is particularly useful for the delivery of drugs to solid nervous tissue.


[0006] CED is capable of obtaining in situ drug concentrations several orders of magnitude greater than those achieved by systemic administration. The concentration profile is relatively flat up to the flow front, providing control over undesired toxicity [Paul F. Morrison, Douglas W Laske, Hunt Bobo. High-flow microinfusion: tissue penetration and pharmodynamics. Am. J. Physiol. 266: 292-305, 1994].


[0008] Another therapeutic method is photodynamic therapy (PDT). At the first step of treatment, one or more drugs that bind to rapidly dividing cells are administered either directly to a tissue or organ or systematically to the treated subject. The drugs administered for PDT are commonly known as photosensitizers due to their inherent ability to absorb photons of light and transfer that energy to oxygen which then converts to a cytotoxic or cytostatic species. Approximately 24-48 hours after the injection, a narrow-band laser is used to excite the photosensitive drug, inducing a chemical reaction which results in a production of free radicals and/or other reactive products that destroy the abnormal tissue or cell with relatively small damage to the surrounding healthy tissue.

[0009] An additional technique, commonly known as electrosurgery, involves the applying of electrical energy to structures in the treated organ. In this technique, an electrosurgical probe or catheter is positioned adjacent the target site. High frequency voltage is then applied to the electrosurgical probe or catheter to volumetrically remove or ablate at least a portion of the structure in situ. This method is particularly useful for volumetrically removing arteriomatic or thrombotic occlusions in blood vessels, or tumors in the brain.

[0010] In another type of treatments, generally referred to as gene therapy, genes or gene-related products (such as RNA) are delivered into the target site for the purpose of genetic therapy of genetic-related disorders. The gene therapeutic is typically delivered via viral vectors, naked DNA, plasmid DNA, liposome-DNA complexes and the like.

[0011] Irrespective of the type of treatment, it is recognized that early assessment of the response of the tissue to the
treatment is of utmost importance. In particular when the treated site is near or in the brain where it is desired to selectively destroy the unwanted tissue while sparing neighboring healthy tissues.

[0012] There is thus a widely recognized need for, and it would be highly advantageous to have a method and system for monitoring cytotoxic response of tissue during or subsequently to a treatment of the tissue by chemicals, genes, thermal treatments or other destructive methods.

SUMMARY OF THE INVENTION

[0013] According to one aspect of the present invention there is provided a method of determining a response of a tissue to a destructive treatment. The method comprises providing magnetic resonance images of the tissue, and using the magnetic resonance images for determining the response of the tissue to the destructive treatment.

[0014] According to still further features in the described preferred embodiments the method further comprises imaging the tissue.

[0015] According to further features in preferred embodiments of the invention described below, regions containing tissues being responsive to the destructive treatment are distinguish from regions containing other tissues.

[0016] According to still further features in the described preferred embodiments the destructive treatment comprises a convection-enhanced drug delivery.

[0017] According to still further features in the described preferred embodiments the destructive treatment comprises a systemic chemotherapy.

[0018] According to still further features in the described preferred embodiments the destructive treatment comprises destruction by gene therapy or gene-related therapy.

[0019] According to still further features in the described preferred embodiments the destructive treatment comprises a thermal treatment.

[0020] According to still further features in the described preferred embodiments the destructive treatment comprises cryotherapy.

[0021] According to still further features in the described preferred embodiments the destructive treatment comprises ablation.

[0022] According to still further features in the described preferred embodiments the destructive treatment comprises heat ablation.

[0023] According to still further features in the described preferred embodiments the destructive treatment comprises ultrasound ablation.

[0024] According to still further features in the described preferred embodiments the destructive treatment comprises RF ablation.

[0025] According to still further features in the described preferred embodiments the destructive treatment comprises a photodynamic therapy.

[0026] According to still further features in the described preferred embodiments the destructive treatment comprises an electrosurgical treatment.

[0027] According to still further features in the described preferred embodiments the imaging is effected by diffusion-weighted magnetic resonance imaging.

[0028] According to still further features in the described preferred embodiments the imaging is effected by T2-weighted magnetic resonance imaging.

[0029] According to still further features in the described preferred embodiments the method further comprises administering at least one MRI contrast agent to the tissue prior to the imaging of the tissue.

[0030] According to still further features in the described preferred embodiments the method further comprises repeating the imaging of the tissue a plurality of times so as to monitor the response of the tissue over time.

[0031] According to still further features in the described preferred embodiments the imaging of the tissue and the determining of the response is done substantially in real time.

[0032] According to another aspect of the present invention there is provided a method of monitoring convection during direct convective interstitial infusion. The method comprises providing a pharmaceutical composition which comprises a therapeutic agent and an MRI contrast agent. The pharmaceutical composition is in liquid form. The method further comprises pressuring the pharmaceutical composition through at least one direct convective interstitial infusion catheter into an interstitial volume of a tissue, imaging a region containing the tissue by MRI, and using the obtained MR image(s) for monitoring the convection of the therapeutic agent within the tissue.

[0033] According to further features in preferred embodiments of the invention described below, the monitoring of the convection comprises comparing the images with images acquired prior to the treatment.

[0034] According to still further features in the described preferred embodiments the convection monitoring comprises correlating an intensity level of the images with a level of presence of the therapeutic agent.

[0035] According to still further features in the described preferred embodiments the convection monitoring comprises correlating an intensity level of the images with a concentration of the therapeutic agent.

[0036] According to still further features in the described preferred embodiments the convection monitoring comprises calculating distribution volume of the pharmaceutical composition.

[0037] According to still further features in the described preferred embodiments the convection monitoring comprises calculating expansion rate of the pharmaceutical composition.

[0038] According to still further features in the described preferred embodiments the convection monitoring comprises detection backflow of the pharmaceutical composition along one or more of the direct convective interstitial infusion catheters. According to still further features in the described preferred embodiments the convection monitoring comprises leakage of the pharmaceutical composition into other low resistance paths, such as, but not limited to, necrotic regions or regions of liquid accumulation. The monitoring can also comprise convection along white matter tracks in the brain or along borders between different types of tissues (e.g. muscle, bone, skin, and the like) in other body organs.

[0039] According to still further features in the described preferred embodiments the convection monitoring comprises detection backflow of the pharmaceutical composition along one or more of the direct convective interstitial infusion catheters. According to still further features in the described preferred embodiments the convection monitoring comprises leakage of the pharmaceutical composition into other low resistance paths, such as, but not limited to, necrotic regions or regions of liquid accumulation. The monitoring can also comprise convection along white matter tracks in the brain or along borders between different types of tissues (e.g. muscle, bone, skin, and the like) in other body organs.

[0040] According to still further features in the described preferred embodiments the imaging is effected by T1-weighted MRI.

[0041] According to still further features in the described preferred embodiments the method further comprises provid-
ing diffusion-weighted or T2-weighted magnetic resonance images of the tissue, and using the diffusion-weighted or T2-weighted magnetic resonance images for determining the response of the tissue to the direct convective interstitial infusion.

[0042] According to still further features in the described preferred embodiments the response of the tissue is determined by calculating apparent diffusion coefficient (ADC) of the tissue and correlating the ADC with the response of the tissue. The response of the tissue is preferably determined by comparing baseline ADC maps, acquired prior to the treatment, to the ADC maps acquired during or subsequently to the treatment.

[0043] According to still further features in the described preferred embodiments the determining the response of the tissue comprises generating an ADC map of the treated tissue and of neighboring tissues.

[0044] According to still further features in the described preferred embodiments the tissue is a tumor, a brain pathology, a spine pathology or any other pathology in other body organs.

[0045] According to yet another aspect of the present invention there is provided a system for determining a response of a tissue to a destructive treatment, the system comprises an MRI apparatus for providing magnetic resonance images of the tissue, and an image processing unit for processing the magnetic resonance images so as to determine the response of the tissue to the destructive treatment.

[0046] According to still further features in the described preferred embodiments the response of the tissue comprises a cytotoxic response.

[0047] According to still further features in the described preferred embodiments the response of the tissue comprises a necrotic response.

[0048] According to still further features in the described preferred embodiments the response of the tissue comprises an inflammatory response.

[0049] According to still further features in the described preferred embodiments the MRI apparatus and the image processing unit are designed and configured to distinguish regions containing tissues being responsive to the destructive treatment from regions containing other tissues.

[0050] According to still further features in the described preferred embodiments the system further comprises a destructive treatment apparatus.

[0051] According to still further features in the described preferred embodiments the destructive treatment apparatus comprises at least one direct convective interstitial infusion catheter.

[0052] According to still further features in the described preferred embodiments the destructive treatment apparatus comprises a unit for destroying cells by systemic chemotherapy.

[0053] According to still further features in the described preferred embodiments the destructive treatment apparatus comprises a unit for destroying cells by gene therapy or gene-related therapy.

[0054] According to still further features in the described preferred embodiments the destructive treatment apparatus comprises a unit for destroying cells by thermal treatment.

[0055] According to still further features in the described preferred embodiments the destructive treatment apparatus comprises a unit for destroying cells by cryotherapy.

[0056] According to still further features in the described preferred embodiments the destructive treatment apparatus comprises a heat ablation unit.

[0057] According to still further features in the described preferred embodiments the destructive treatment apparatus comprises a unit for ablating tissues.

[0058] According to still further features in the described preferred embodiments the destructive treatment apparatus comprises an ultrasound ablation unit.

[0059] According to still further features in the described preferred embodiments the destructive treatment apparatus comprises an RF ablation unit.

[0060] According to still further features in the described preferred embodiments the destructive treatment apparatus comprises a photodynamic therapy unit.

[0061] According to still further features in the described preferred embodiments the destructive treatment apparatus comprises an electrosurgical treatment unit.

[0062] According to still further features in the described preferred embodiments the image processing unit calculates ADC of the tissue and correlates the ADC with the response of the tissue. According to still further features in the described preferred embodiments the image processing unit generates an ADC map of the tissue and of neighboring tissues.

[0063] According to still another aspect of the present invention there is provided a system for monitoring convective during direct convective interstitial infusion. The system comprises: an MRI apparatus; one or more direct convective interstitial infusion catheters for delivering one or more pharmaceutical compositions as described herein; and an image processing unit for processing the magnetic resonance images so as to monitor the convection of the therapeutic agent within the tissue.

[0064] According to further features in preferred embodiments of the invention described below, the image processing unit compares the images with images acquired prior to the treatment.

[0065] According to still further features in the described preferred embodiments the image processing unit correlates an intensity level of the images with a level of presence of the therapeutic agent.

[0066] According to still further features in the described preferred embodiments the image processing unit correlates an intensity level of the images with a concentration of the therapeutic agent.

[0067] According to still further features in the described preferred embodiments the image processing unit detects backflow of the pharmaceutical composition along the direct convective interstitial infusion catheter(s). According to still further features in the described preferred embodiments image processing unit detects leakage of the pharmaceutical composition into other low resistance paths, such as, but not limited to, necrotic regions or regions of liquid accumulation.
According to still further features in the described preferred embodiments, the magnetic resonance images comprise any magnetic resonance images suitable for detecting the contrast agent, including, without limitation, T1-weighted magnetic resonance images.

According to still further features in the described preferred embodiments, the system further comprises the pharmaceutical composition.

According to still further features in the described preferred embodiments, the pharmaceutical composition comprises a nanoparticle. According to still further features in the described preferred embodiments, the nanoparticle comprises iron oxide.

According to still further features in the described preferred embodiments, the at least one MRI contrast agent comprises a paramagnetic metal.

According to still further features in the described preferred embodiments, the at least one MRI contrast agent comprises a superparamagnetic metal.

According to still further features in the described preferred embodiments, the at least one MRI contrast agent comprises a ferromagnetic paramagnetic metal.

According to still further features in the described preferred embodiments, the at least one MRI contrast agent has a T1 shortening effect.

According to still further features in the described preferred embodiments, the at least one MRI contrast agent has a T2 shortening effect.

According to still further features in the described preferred embodiments, the at least one MRI contrast agent comprises a diethylenetriamine pentaacetic acid (DTPA).

According to still further features in the described preferred embodiments, the paramagnetic metal comprises Gadolinium (Gd).

According to still further features in the described preferred embodiments, the at least one MRI contrast agent comprises Gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA).

According to an additional aspect of the present invention, there is provided a method for generating a catheter acceptance map for convection-enhanced drug delivery into an organ, such as, but not limited to, a brain or a prostate. The method comprises providing at least one image of the organ, analyzing the image(s) so as to identify different regions corresponding to different catheter acceptance levels for efficient convection, and marking the regions so as to allow distinction between the different regions, thereby generating the catheter acceptance map.

According to a further aspect of the present invention, there is provided a catheter acceptance map of an organ, comprising at least one image of the organ having distinctly marked regions, each corresponding to a different catheter acceptance levels for efficient convection.

The present invention successfully addresses the shortcomings of the presently known configurations by providing systems and methods for monitoring convection and determining a response of a tissue to a destructive treatment. The systems and methods of the present invention enjoy properties far exceeding the prior art.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particular shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

FIG. 1 is a schematic illustration of a system for determining a response of a tissue to a destructive treatment, according to various exemplary embodiments of the present invention;

FIG. 2 is a flowchart of a method for determining a response of a tissue to a destructive treatment, according to various exemplary embodiments of the present invention;

FIG. 3 is a flowchart diagram of the convection monitoring method, according to various exemplary embodiments of the invention;

FIG. 4 is a schematic illustration of a system for monitoring convection during direct convective interstitial infusion, according to various exemplary embodiments of the invention;

FIG. 5A is a flowchart diagram of a method for generating a catheter acceptance map, according to various exemplary embodiments of the present invention;

FIG. 5B is a schematic illustration of a catheter acceptance map, according to various exemplary embodiments of the present invention;

FIGS. 6A-B are T1 and T2-weighted axial MR images of normal rat brain;

FIGS. 6C-E are T1-weighted MR images acquired immediately post CED treatment with infusates containing Gd-DTPA and Evans Blue, showing poor (FIG. 6C), moderate (FIG. 6D) and efficient (FIG. 6E) convective efficiency;

FIGS. 6F-H show fixated brain samples of the same rats of FIGS. 6C-E, demonstrating similar distributions of the Evans Blue dye in the tissue;

FIGS. 7A-C are diffusion-weighted MR images of rats treated with Taxol® (FIG. 7A), Cremophor (FIG. 7B) and Carboplatin (FIG. 7C);

FIG. 8A is a T1-weighted MR image, acquired immediately following CED of Taxol® mixed with Gd-DTPA;

FIGS. 8B-C are T2-weighted (FIG. 8B) and diffusion-weighted (FIG. 8C) MR images of the same rat as FIG. 8A;

FIG. 8D is a low-power magnification of an en block-resected lesion taken from the rat brain of FIGS. 8A-C;

FIGS. 9A1-A3 are T1-weighted MR images acquired immediately post treatment with Gd-DTPA mixed
with: 0.2% human serum albumin (FIG. 9A1), 17% sucrose (FIG. 9A2) and Taxol (FIG. 9A3);

[0099] FIGS. 9B1-B3 are T1-weighted MR images acquired 24 hours post treatment with Gd-DTPA mixed with: 0.2% human serum albumin (FIG. 9B1), 17% sucrose (FIG. 9B2) and Taxol (FIG. 9B3);

[0100] FIGS. 9C1-C3 are T2-weighted MR images acquired 24 hours post treatment with Gd-DTPA mixed with: 0.2% human serum albumin (FIG. 9C1), 17% sucrose (FIG. 9C2) and Taxol (FIG. 9C3);

[0101] FIGS. 9D1-D3 are T1-weighted MR acquired 24 hours post treatment with Gd-DTPA mixed with: 0.2% human serum albumin (FIG. 9D1), 17% sucrose (FIG. 9D2) and Taxol (FIG. 9D3);

[0102] FIGS. 10A-C show correlation between enhancement volume in T1-weighted MRI and enhancement volume in diffusion-weighted MRI; and
[0103] FIGS. 11A-B are gradient echo MR images acquired immediately post treatment with low viscosity (FIG. 10) and high viscosity (FIG. 11) of infusate containing iron oxide nanoparticles, in accordance with preferred embodiments of the present invention;

[0104] FIG. 12A shows backflow into the ventricles as depicted by gradient-echo MRI immediately post a CED treatment with a 0.2 mg/ml infusate at 8 μl/min over 7.5 min; 
[0105] FIG. 12B shows efficient IO distribution as depicted by gradient-echo MRI immediately post a CED treatment with a 0.2 mg/ml infusate at 4 μl/min over 15 minutes;
[0106] FIG. 12C shows efficient IO distribution as depicted by direct visualization immediately post treatment with a high concentration infusate;
[0107] FIG. 12D shows efficient fluorescent-labeled IO distribution as depicted by spectral imaging immediately post treatment;
[0108] FIGS. 13A-D show gradient-echo MR images of a rat taken immediately (FIG. 13A), 3 days (FIG. 13B), 6 days (FIG. 13C) and 27 days (FIG. 13D) post a CED treatment with a 0.2 mg/ml infusate at 4 μl/min over 15 minutes;
[0109] FIGS. 14A-D show gradient-echo MR images of another rat, taken immediately (FIG. 14A), 3 days (FIG. 14B), 6 days (FIG. 14C) and 27 days (FIG. 14D) post a CED treatment with a 0.2 mg/ml infusate at 4 μl/min over 15 minutes;
[0110] FIGS. 15A-C show gradient-echo MR images of three rats taken immediately post treatment with low concentration IO in 20% sucrose solution at infusion rates of 1 μl/min over 50 minutes (FIGS. 15A-B), and 2 μl/min over 15 minutes (FIG. 15C);
[0111] FIGS. 16A-H show an example of a large C6 tumor induced in the thigh of a babble-c mouse treated by CED of high concentration Paclitaxel, where FIGS. 16A-B are T2 MR images of the tumor prior to treatment, FIGS. 16C-D are T1 MR images taken immediately post treatment, FIGS. 16E-G are T2 MR images taken 24 hours post treatment, and FIG. 16H is an image of the treated mouse;
[0112] FIGS. 17A-M show an example of a large C6 tumor induced in the thigh of another babble-c mouse treated by CED of high concentration Paclitaxel, where FIGS. 17A-D are T2 MR images of the tumor prior to treatment, FIG. 17E-H are T1 MR images taken immediately post treatment, FIG. 17I-L, are T2 MR images taken 24 hours post treatment, and FIG. 17M is an image of the treated mouse;
[0113] FIGS. 18A-22B are T1-weighted MR images (A) and respective optical images (B) of brains of five different rats, treated by CED with high viscosity infusates containing blue bovine serum albumin and Gd-DTPA;

[0114] FIGS. 23A-H are MR images acquired immediately (FIGS. 23A-D) and 24 hours (FIGS. 23E-H) post treatment in non-specific neurotoxicity experiments where different solvents were used for dissolving Taxol; and
[0115] FIG. 24 is a flowchart diagram of a method suitable for preparing a pharmaceutical composition with minimal non-specific toxicity for direct convective interstitial infusion, according to various exemplary embodiments of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0116] The present invention is of a method and system which can be used for monitoring convection and/or determining a response of a tissue to convection-based or other destructive treatments. Specifically, the present invention can be used to monitor the convection and/or determine the response of a tissue using magnetic resonance imaging (MRI).

[0117] The principles and operation of a method and system according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

[0118] Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

[0119] MRI is a method to obtain an image representing the chemical and physical microscopic properties of materials, by utilizing a quantum mechanical phenomenon, named Nuclear Magnetic Resonance (NMR), in which a system of spins, placed in a magnetic field resonantly absorb energy, when applied with a certain frequency.

[0120] When placed in a magnetic field, a nucleus having a spin 1 is allowed to be in a discrete set of energy levels, the number of which is determined by I, and the separation of which is determined by the gyromagnetic ratio γ of the nucleus and by the magnetic field. Under the influence of a small perturbation, manifested as a radiofrequency magnetic field rotating about the direction of a static magnetic field, the nucleus has a time-dependent probability to experience a transition from one energy level to another. With a specific frequency of the rotating magnetic field, the transition probability may reach the value of unity. Hence, at certain times a transition is forced on the nucleus, even though the rotating magnetic field may be of small magnitude relative to the primary magnetic field. For an ensemble of spin 1 nuclei, the transitions are realized through a change in the overall magnetization.

[0121] Once a change in the magnetization occurs, a system of spins tends to restore its magnetization longitudinal equilibrium value, in accordance with the thermodynamic principle of minimal energy. The time constant which control the elapsed time for the system to return to the equilibrium value is called "spin-lattice relaxation time" and is denoted T1. An additional time constant, T2 (<T1), called "spin-spin relaxation time", controls the elapsed time in which the transverse
magnetization diminishes, in accordance with the thermodynamic principle of minimal to energy. However, inter-molecular interactions and local variations in the value of the static magnetic field alter the value of T2 to an actual value denoted T2*.

In MRI, a static magnetic field having a predetermined gradient is applied on an object, thereby creating, at each region of the object, a unique magnetic field. By detecting the NMR signal, knowing the magnetic field gradient, the position of each region of the object can be imaged. Magnetic resonance (MR) pulse sequences applied to the object (e.g., a patient) induce, refocus and/or manipulate the magnetic resonance by interacting with the spins. NMR signals are generated and used for obtaining information and reconstructing images of the object. The above mentioned relaxation times and the density distribution of the nuclear spins are properties which vary from one normal tissue to the other, and from one diseased tissue to the other. These quantities are therefore responsible for contrast between tissues in various imaging techniques, hence permitting image segmentation.

Many MR sequences are known. Broadly speaking the various time instants of the MR sequences are selected so as to encode the magnetic resonance to provide spatial information, flow information, diffusion information and the like.

In diffusion-weighted MRI, for example, the magnetic field gradients are selected so as to provide motion-related contrast which is sensitive to motion of fluid molecules in selected directions. Diffusion-weighted MRI exploits the random motion of the molecules which causes a phase dispersion of the spins with a resultant signal loss.

The MR sequence is selected so as to control the T2 relaxation process, and to minimize T1 effect. One method for such control is called the spin-echo method, in which the magnetization is first forced to lie in the transverse plane and, after a predetermined time-interval, refocused by a 180° flip. The peaks of the resulting signal are described by a decay curve characterized by the T2 time-constant.

The present invention exploits the advantages of MRI for monitoring progress of various destructive treatments and determining tissue response to the treatments.


However, Mardor et al. and Lidar et al. have only focused on one type of treatment, (CED of Taxol®), and did not use diffusion-weighted MRI in combination with any other destructive treatment. Furthermore, neither Mardor et al. nor Lidar et al. realized that the diffusion-weighted MR images can be used to determine cytotoxic response of the tissue.

It is therefore the object of the present invention to provide a system and method for determining tissue response to many destructive treatments, preferably, but not exclusively, destructive treatments which are other than CED of Taxol®. Representative examples of such destructive treatments which can be used in various exemplary embodiments of the invention include, without limitation, systemic chemotherapy, thermal treatment, ablation (e.g., heat ablation), cryotherapy, photodynamic therapy, electrosurgical treatment. Also contemplated is the use any type of convective-based treatments, such as, but not limited to, CED.

Referring now to the drawings, FIG. 1 illustrates a system 10 for determining a response of a tissue 12 to a destructive treatment. System 10 preferably comprises an MRI apparatus 14, for providing MR images of tissue 12. The MR images can be diffusion-weighted MR images or T2-weighted MR images. In any event, the type of MR image is preferably selected so as to allow exclusive determination of the tissue response to the destructive treatment. The imaging of the tissue can be repeated more than one time so as to monitor the response of the tissue over time.

The advantage of using diffusion-weighted MRI is that such technique allows accurate control of the diffusion direction and the time during which diffusion takes place. Additionally, diffusion-weighted MRI enables the determination of the mean diffusion path length. MRI sequences suitable for diffusion-weighted MRI are known in the art, e.g., Stejskal ET O and Tanner JE, “Spin diffusion: Spin echoes in the presence of a time-dependent field gradient”, J Chem Phys 42:288-292, 1965. As demonstrated in the Examples section that follows, the use of diffusion-weighted MRI allows exclusive detection of tissues developing cytotoxic response to the destructive treatment. Furthermore, the use of diffusion-weighted MRI allows differentiating between various response and toxicity patterns, such as, but not limited to, global, focal (typically observed as enhanced regions originating close to the catheter tip or track), cortical, gyral, diffuse, necrotic (typically characterized by high ADC) versus inflammatory (typically characterized by low ADC) and the like.

MR sequences suitable for T2-weighted MRI include fast spin-echo and other MR sequences such as, but not limited to, the MR sequences found in Friedburg H and Bockenheimer S, “Clinical NMR tomography with sequential T2 images (Carr-Purcell spin-echo sequences)”, Radiology, 1983, 23(8):353-6.

In various exemplary embodiments of the invention system 10 comprises a destructive treatment apparatus 16, for treating tissue 12. Apparatus 16 can be any apparatus or device capable of destructing tissues.

In preferred embodiments in which direct convective interstitial infusion is employed, apparatus 16 comprises one or more direct convective interstitial infusion catheters. This embodiment is particularly useful when the destructive treatment is by CED.

In preferred embodiments in which chemotherapy is employed, apparatus 16 comprises a unit for destructing cells by systemic chemotherapy. Such unit can be, for example, an infusion unit with chemotherapy drugs. Alternatively or additionally, the drugs can be taken by the subject orally or by a catheter. Similar units can also be used for destructing cells by gene therapy or gene-related therapy.

In preferred embodiments in which thermal treatment is employed, apparatus 16 comprises a unit for destruct-
ing cells by thermal treatment. Thermal treatment units are known in the art. Thermal treatment units suitable to be used in the presently preferred embodiment of the invention are found, for example, in U.S. Pat. Nos. 5,995,875, 6,106,521, 6,579,285, 6,605,084, 6,618,620, 6,635,054, 6,764,485, 6,740,108 and 6,605,082, the contents of which are hereby incorporated by reference.

[0137] In preferred embodiments in which ablation is employed, apparatus 16 comprises an ablation unit, such as, but not limited to, a heat ablation unit which locally heats tissue 12 to ablate it or at least cause its destruction. The energy for ablating and/or destroying the tissue can be transferred to the tissue by any way known in the art, such as, but not limited to, light (laser, infrared, etc.), ultrasound, radiofrequency waves and the like. Other ablation or thermal treatments are also contemplated. For example, in an alternatively preferred embodiment, apparatus 16 destruct the cells by cryotherapy. In this embodiment, apparatus 16 can comprises a cryoprobe or a similar device, such as, but not limited to, the systems disclosed in U.S. Pat. Nos. 6,706,037, 6,629,417, 6,251,105 and 6,017,373, the contents of which are hereby incorporated by reference.

[0138] In preferred embodiments in which photodynamic therapy is employed, apparatus 16 comprises a photodynamic therapy unit. Such unit may include a photon emitting device and, optionally and preferably, means for administering photosensitive drug. In this embodiment, the subject is preferably administered by the photosensitive drug. Following the administration, the photon emitting device excites the photosensitive drugs and induces the aforementioned production of free radicals and/or other reactive products that destroy tissue 12. Photodynamic therapy units suitable to be used in the presently preferred embodiment of the invention are found, for example, in U.S. Pat. Nos. 4,693,556, 5,344,434, 5,519, 534 and 6,743,249, the contents of which are hereby incorporated by reference.

[0139] In preferred embodiments in which electro surgical treatment is employed, apparatus 16 comprises an electro surgical treatment unit, which can be an electro surgical probe or an electro surgical catheter. Electro surgical treatment units suitable to be used in the presently preferred embodiment of the invention are found, for example, in U.S. Pat. Nos. 6,045, 532, 6,322,549, 6,712,813 and 6,740,079, the contents of which are hereby incorporated by reference.

[0140] The MRI contrast agent can be either a positive or a negative MRI contrast agent.

[0141] As used herein, “positive MRI contrast agent” refers to an agent which increases the signal of the pharmaceutical composition relative to the nearby tissues of fluids, and “negative MRI contrast agent” refers to an agent which decreases the signal of the pharmaceutical composition relative to the nearby tissues of fluids.

[0142] In any event, the MRI contrast agent is preferably selected so as to allow convection of the MRI contrast agent together with the convective wave of the therapeutic agent.

[0143] In various exemplary embodiments of the invention positive MRI contrast agents are used such that their dominant effect is to reduce the T1 relaxation time. In other exemplary embodiments of the invention negative MRI contrast agents are used such that their dominant effect is to reduce the T2 relaxation time.

[0144] In any event, as will be appreciated by one ordinarily skilled in the art, the pharmaceutical composition is distinguished from its surroundings either by an enhanced or reduced NMR signal.

[0145] The magnetic properties of the MRI contrast agent can be of any type. More specifically, the MRI contrast agent comprises a magnetic material which can be paramagnetic, superparamagnetic or ferromagnetic material.

[0146] The magnetic properties of the MRI contrast agent (and all other materials in nature) originate from the subatomic structure of the material. The direction as well as the magnitude of the magnetic force acting on the material when placed in a magnetic field is different for different materials. Whereas the direction of the force depends only on the internal structure of the material, the magnitude depends both on the internal structure as well as on the size (mass) of the material. Ferromagnetic materials have the largest magnetic susceptibility compared to para- or superparamagnetic materials. Superparamagnetic materials consist of individual domains of elements that have ferromagnetic properties in bulk. Their magnetic susceptibility is larger than that of the paramagnetic but smaller than that of ferromagnetic materials.

[0147] Broadly speaking, ferromagnetic and superparamagnetic MRI contrast agents are negative MRI contrast agents and paramagnetic MRI contrast agents can be either negative or positive MRI contrast agents. The effect of paramagnetic material on the magnetic resonance signal depends on the type and concentration of the paramagnetic material, as well as on external factors, such as the strength of the applied magnetic field. In various exemplary embodiments of the invention the MRI contrast agents which comprise paramagnetic materials are positive contrast agents.

[0148] Paramagnetic materials, as used herein, refers to metal atoms or ions which are paramagnetic by virtue of one or more unpaired electrons, and excludes radioactive metal atoms or ions commonly referred to as radionuclides. Representative examples include, without limitation, the paramagnetic transition metals and lanthanides of groups 1b, 2b, 3a, 3b, 4a, 4b, 5b, 6b, 7b, and 8, more preferably those of atomic number 21-31, 39-50, 57-71, and 72-82, yet more preferably gadolinium (Gd), dysprosium (Dy), chromium (Cr), iron (Fe), and manganese (Mn), still more preferably Gd, Mn, and Fe, and most preferably Gd.

The MRI contrast agent preferably comprises a chelating moiety, capable of forming chelate-complexes with the paramagnetic material. These can be linear chelating moieties such as, but not limited to, polyamino polyethylene polynacetic acids e.g., diethylenetramine pentaacetic acid (DTPA), ethylene diamine tetraacetic acid (EDTA), triethylene tetramine hexaacetic acid (TESHA) and tetracyclene pentamethine heptacetic acid), or cyclic chelating moieties such as, but not limited to, polyazacyclacetacetic compounds e.g., such as 1,4,7,10-tetraacyclododecane-1,4,7,10-tetraacetic acid (DOTA).

In various exemplary embodiments of the invention the MRI contrast agent is a positive MRI contrast agent which comprises Gd-DTPA. Gd-DTPA is a positive contrast agent when observed via T1-weighted MRI and a negative contrast agent when observed via T2-weighted MRI. As T1 is more sensitive to Gd-DTPA, T1-weighted MRI is the preferred MRI technique when the contrast agent is Gd-DTPA.

Also contemplated are MRI contrast agents in a form of nanoparticles, such as, but not limited to, iron oxide nanoparticles.

The above MRI contrast agents can be formulated (e.g., mixed) with many therapeutic agents or solvents, include, without limitation, Cremophor, TaloXol®, Carboplatin, Ethanol, sugars, human serum albumin, dimethyl sulfoxide and Dextane. Also contemplated are agents which are presently used in animal as well as phase I, II and III of clinical studies for convection-based therapy of brain tumors and other brain pathologies. Representative examples include, without limitation, agents known as: IL13-PE38QQR, TP-38, IL4(38-37)-PE38 KDEL, cplL4-PE, IL-4 cytokotox, IL-4 Pseudomonas exotoxin (NBI-3001), TransMID, TF-CRM107, HSV-1-4k gene-bearing liposomal vector, IL-12, ISFV-IL-12, ST-DTP, DAB389EGF, gemcitabine, carboplatin, glucocerebrosidase, AAV2-TK, LIPO-HSV-1tk gene transfer system, TRAIL, BD-EGF and IMC-C225 (as molecular targeting agents for boron neutron capture therapy), interleukin (IL)-beta and interferon (IFN)-gamma, HAMLET, 6-hydroxydopamine, targeted chimera cytotoxic proteins, AmC, AAV-2, topotecan, double-stranded RNAdependent protein kinase PKR, and AP20398. Some of these agents are presently in animal as well as phase I, II and III of clinical studies for convection-based therapy of brain tumors and other brain pathologies.

In step 34 of the method the pharmaceutical composition is pressured into an interstitial volume of the tissue using one or more direct convective interstitial infusions catheters. The method continues to step 36 in which a region containing the tissue is imaged by MRI. Many MRI pulse sequences can be used for acquiring the MR images. Typically, the pulse sequence is compatible with the type of MRI contrast agent present in the pharmaceutical composition.

Thus, for positive MRI contrast agents having a T1 shortening effect, the pulse sequence is preferably a T1-weighted pulse sequence, and for negative MRI contrast agents, having a T2 shortening effect, the pulse sequence is preferably a T2-weighted or T2*-weighted pulse sequence.

Once the region is imaged, the method continues to step 38 in which the images are used for monitoring the convection of the therapeutic agent within the tissue. According to the presently preferred embodiment of the invention the monitoring is by comparing the images with images acquired prior to the treatment. Additionally or alternatively, the intensity of the image or the contrast spread can be correlated with the presence of the therapeutic agent. This can be done, for example, by defining region-of-interests, and multiplying the number of pixels in the region-of-interests with the volume represented by a single pixel. As will be appreciated by one of ordinary skill in the art, such procedure can be used to define the distribution volume, expansion rate and/or direction of expansion of pharmaceutical composition. The determination of presence of the pharmaceutical composition can also be used for detecting backflow of the pharmaceutical composition along the catheter or leakage into a low resistance path such as, but not limited to, necrotic regions or regions of liquid accumulation, stopping or preventing convection formation. This information is particularly useful, for example, for the purpose of real time treatment adjustments.

The method ends at step 39.

The convection monitoring of the presently preferred embodiment of the invention can be performed by a system 40 for monitoring convection during direct convective interstitial infusion.

Reference is now made to FIG. 4, which is a schematic illustration of system 40, according to a preferred embodiment of the present invention. System 40 preferably comprises an MRI apparatus (e.g., apparatus 14 above), for providing magnetic resonance images using an appropriate pulse sequence, as further detailed hereinabove. System 40 further comprises one or more direct convective interstitial infusion catheters 42 for delivering the pharmaceutical composition to the interstitial volumes of the tissue. Catheter 42 can be any known catheter capable of direct interstitial delivery, such as, but not limited to, the catheters disclosed in U.S. Pat. Nos. 6,758,828, 6,824,532 and 6,368,315 and U.S. Patent Application No. 20040199128. The pharmaceutical composition can be held in a container 46 which is in fluid communication with catheter 42. Catheter 42 and/or container 46 can be connected to a pump 47 for providing the necessary pressure of composition to catheter 42.

System 40 further comprises an image processing unit 44 for processing the MR images so as to monitor the convection of the therapeutic agent within the tissue. Image processing unit 44 preferably monitors the convection by: (i) comparing the images with baseline images acquired prior to treatment; (ii) correlating the intensity level of the images with the presence of the therapeutic agent; and/or (iii) calculating distribution volume, as further detailed hereinabove.
several animals. The treatment efficacy can be determined by obtaining MR images immediately post treatment and a pre-determined time-period (e.g., several hours or days) thereafter.

[0163] Assessment of CED formation and extent can then be performed, for example, by obtaining T1-weighted MR images immediately post treatment, and calculating the distribution volume of the composition. The calculation can be performed by defining regions-of-interests over the entire enhanced region of the images, and multiplying the number of pixels in the regions-of-interests by the volume which corresponds to a single pixel.

[0164] Early assessment of cytotoxic tissue response can be performed, for example, by obtaining T2-weighted and/or diffusion-weighted MR images immediately or even early after the treatment (for example, 24 hours after the treatment).

[0165] In case of non-specific cytotoxic tissue response to the drug, the formulation can be changed (manipulate the drug solvents or other physical, chemical, biological characteristics of the composition to decrease the cytotoxicity) and the above procedure can be repeated in an additional group of animals until cytotoxic tissue response is reduced to an acceptable level.

[0166] In cases of inefficient CED formation, the viscosity of the composition can be increased and the above procedure can be repeated in an additional group of animals, until the satisfactory distributions of drug are reached.

[0167] An exemplified protocol for formulation of composition for CED is provided in the Examples section that follows.

[0168] The present embodiments can also be used for generating a catheter acceptance map for CED in an organ, such as the brain, the prostate and the like.

[0169] A CED procedure is typically preceded by planning the catheter positioning using a software package (e.g., BrainLab™, Medtronic™, etc.). Conventional packages analyze images (typically CT or MR images) of the brain and provide three-dimensional information of the anatomy of the brain based on the analyzed images. However, although conventional packages allow to define specific catheter tracks, these packages do not provide information regarding the sensitivity of convection formation in different regions of the brain.

[0170] Other software packages (such as the simulation package developed by Brainlab™), are capable of predicting spatial and temporal distributions of convection by combining simulation technique and MRI information, including sequences such as diffusion weighted MRI, diffusion tensor MRI (DTI) and perfusion MRI. This software is typically used after planning a certain catheter track so as to predict the convective distribution.

[0171] The teachings of the present invention allow the construction of a catheter acceptance map for CED which comprises information regarding the acceptance levels of the catheter in different regions of the organ. The catheter acceptance map of the present embodiment can present the organ in terms of appropriateness for convection catheter tracks. This allows the operator or neurosurgeon to easily plan catheter tracks in the organ. Furthermore, even when the operator faces a situation in which the actual catheter track deviates from the desired track, the map of the present embodiment allows the operator to decide, substantially in real time, whether or not a repositioning of the catheter is necessary. For example, the operator may decide that although the actual track deviates from the original plan, it is still appropriate for forming efficient convection and there is no need for repositioning of the catheter.

[0172] A preferred method for generating such a map is illustrated in the flowchart diagram of FIG. 5A, and a schematic illustration of such catheter acceptance map is illustrated in FIG. 5B.

[0173] Referring to FIG. 5A, the method begins at step 50 and continues to step 52 in which one or more images of the organ are provided. The images can be, for example, MR images (e.g., T1-weighted, T2-weighted or diffusion-weighted MR images), a single photon emission CT (SPECT) image, a positron emission tomography (PET) image, DTI, MR perfusion, CT perfusion and the like. In various exemplary embodiments of the invention the image(s) is/are three-dimensional image(s) such that the generated map is a three-dimensional map.

[0174] The method continues to step 54 in which the image (or images) is analyzed so as to identify different regions in the image, whereby each different region corresponds to a different catheter acceptance level for efficient convection.

[0175] As used herein, “catheter acceptance level” refers to the ability of a tissue or pattern of tissues to receive the CED catheter and to allow efficient convection into the organ. The catheter acceptance level can be defined in a binary manner, or in any other discrete or continuous manner. For example, when the catheter acceptance levels are defined in a binary manner (i.e., there are only two different levels) each region in the map can have a “low” catheter acceptance level, in which case the region is less favored for CED, or a “high” catheter acceptance level in which case the region is more favored for CED. As will be appreciated by one ordinarily skilled in the art, “low” and “high” can be represented numerically by, e.g., “0” and “1”, respectively. Alternatively, more than two catheter acceptance levels can be defined and be assigned with a discrete or numerical value.

[0176] The catheter acceptance level of each picture element (e.g., pixel, group of pixels, voxel, group of pixels) is preferably a weighted combination of several parameters which measure the efficiency of treatment. Such parameters can include, for example, local tissue density at the particular picture element, distances from paths known to have low resistance to convection (e.g., ventricle, sulcus, major white matter track, severe necrosis regions etc.), distances from major blood vessels, perfusion and the like.

[0177] Thus, the regions of the image (and the corresponding regions of the organ) are classified according to their ability to receive the CED catheter and to allow efficient convection into the organ.

[0178] The method proceeds to step 56 in which the different regions are marked in a distinctive manner (e.g., using different colors and/or different gray levels) so as to allow distinction between different regions. A representative example of a CED map 60 is illustrated in FIG. 5B. As shown, map 60 has a plurality of distinctly marked regions 62, where each region corresponds to a different catheter acceptance level. In the representative example of FIG. 5B, regions 62a have low catheter acceptance level, hence being the most inappropriate for CED, regions 62b have medium catheter acceptance level, hence being less favored for CED, and regions 62c have a high catheter acceptance level, hence being the most inappropriate for CED.
by different gray levels, where regions 62a are the brightest and regions 62c are the darkest. Other distinctive markings are also contemplated.

[0179] The method ends at step 58.

[0180] Pharmaceutical compositions suitable for the present embodiments preferably comprise a pharmaceutical agent and a pharmaceutical carrier.

[0181] As used herein, “pharmaceutical composition” refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

[0182] Hereinafter, the phrases “physiologically acceptable carrier” and “pharmaceutical carrier,” which may be used interchangeably, refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

[0183] Herein, the term “excipient” refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Representative examples of excipients include, without limitation, calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

[0184] The composition of the present embodiments is preferably in a liquid form, which can be, a solution, a suspension, an emulsion and the like. Thus, for example, in one embodiment, the pharmaceutical carrier is in a liquid form and the pharmaceutical agent is dissolved or dispersed in the pharmaceutical carrier. In another embodiment, the pharmaceutical agent is in a liquid form and is co-formulated (e.g., mixed) with the pharmaceutical carrier.

[0185] In various exemplary embodiments of the invention the pharmaceutical composition has a sufficiently high viscosity.

[0186] As used herein “sufficiently high viscosity” refers to a viscosity value such that during direct convective interstitial infusion using a properly placed catheter(s), a perfusion of the composition into interstitial volumes of a tissue is ensured with minimal or substantially without backflow.

[0187] The viscosity is conveniently expressed in relative values, in units of saline viscosity or the like. It was found by the inventors of the present invention that CED efficiency (for example, in terms of CED volume) is an increasing function of the viscosity of the composition. Specifically it was found that efficient CED can be obtained when the viscosity is higher than the viscosity of a human serum albumin (HSA) solution at a concentration of about 0.2%. It was also found by the inventors of the present invention that efficient CED can be obtained when the viscosity is higher than the viscosity of sucrose 5%.

[0188] Thus, in various exemplary embodiments of the invention the viscosity is above a predetermined viscosity value, which equals the viscosity of 0.2% HSA, more preferably, more preferably the viscosity of 1.2% HSA, more preferably the viscosity of 2% HSA, more preferably the viscosity of 3% HSA, more preferably the viscosity of 4% HSA, more preferably the viscosity of 5% HSA. When compared to the viscosity of sucrose, the viscosity is above a predetermined viscosity value, which preferably equals the viscosity of sucrose 5%.

[0189] In various exemplary embodiments of the invention the viscosity of the composition is selected to allow a flow rate of above 4 microliters per minute, more preferably 5 microliters per minute, more preferably 8 microliters per minute, more preferably 10 microliters per minute.

[0190] Pharmaceutical compositions of the present embodiments may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[0191] Pharmaceutical compositions for use in accordance with the present embodiments thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations that can be used pharmaceutically.

[0192] The active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution or physiological salt buffer.

[0193] Pharmaceutical carriers which can increase the viscosity include, without limitation, serum albumin, sucrose and methanol. Additionally, when the pharmaceutical composition is in a suspension form, substances that increase the viscosity of the suspension may be used. Such substances include, without limitation, sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the active ingredients, to allow for the preparation of highly concentrated solutions.

[0194] Viscosity may also be increased by binding to the therapeutic agent molecules which significantly increase water adhesion such as Dextrane or Albumin.

[0195] The pharmaceutical compositions of the present embodiments can comprise a therapeutic agent and/or a diagnostic agent. The composition of the present embodiments can also include stem cells, in which case the composition can be used for high dose stem cell transplantation. The high viscosity composition may also protect the stem cells (which are sensitive to the pressure) during the delivery into the target tissue.

[0196] Therapeutic agents include active ingredients which are contained in the pharmaceutical composition is an amount effective to achieve the intended purpose. More specifically, a “therapeutically effective amount” means an amount of active ingredients (e.g., an anti-proliferative agent, a chemotherapeutic agent, a nucleic acid construct) effective to prevent, alleviate, or ameliorate symptoms of a disorder or prolong the survival of the subject being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0197] Many therapeutic agents are contemplated. Representative examples include, without limitation, chemotherapeutic agents (e.g., neurologic agents), anti-inflammatory agents (steroidal and non-steroidal), gene therapy agents, antitumor agents, esthetic agents, antimicrobial agents, anti-inflammatory agents, antidepressants, vitamins, antihistamines, hormones, androgenic compounds, progestin compounds and drug carriers for slow drug release or targeting.

[0198] Examples of chemotherapeutic or chemotherapeutic-related agents include, without limitation, Cremophor, Taxol®, Carboplatin and Ethanol.
Nevertheless, it was found by the Inventors of the present invention that when the composition is used for treating brain tumors, the presence of Cremophor in the composition results in non specific toxic response. Thus, in situations in which Cremophor may result in non specific toxic response (e.g., in brain tumor treatments), the pharmaceutical composition is preferably Cremophor free. Additional examples of chemotherapeutic agents include, without limitation, an alkylating agent such as a nitrogen mustard, an ethyleneimine and a methylmelamine, an alkyl sulfonate, a nitrosourea, and a triazine; an antimetabolite such as a folic acid analog, a pyrimidine analog, and a purine analog; a natural product such as a vinca alkaloid, an organic pyridoxytoxin, an antibiotic, an enzyme, a taxane, and a biological response modifier; miscellaneous agents such as a platinum coordination complex, an anthracenedione, an anthracycline, a substituted urea, a methyl hydrazine derivative, or an adenocortical suppressant. Other examples include, without limitation, BCNU (Carmustine); Imritecan (CPT-11); Cumptotheic (CPT); ipotecan; Oxaliplatin; Carboplatin; Cisplatin (CDDP); CCNU (Lomustine); Doxorubicin; 5'-Fluoro uracil (5FU); Methotrexate; Vinblastine; Vincristine; AS101; Procarbazine (Matulane); etoposide (VP-16); Gemzar (Gemcytobine . . . for pancreas, etc.); Doxil; Navelbine; Cytarabine-A; DepoCyt (in liposomal form); Herceptin; Raloxifene; Tamoxifen; Meace (anti-Progesterone); Somatstatin (pituitary); I-131 (radioactive iodine for thyroid); IL-2; IL-12 (melanoma, renal), cytostan, cyclophosphamide, TS-1, CDDP, rosiglitazone, metformin.

Also contemplated are Taxo®, formulations, as such, as not limited to, ABI-007; CT-2105 (PG-TXL); Fibrogen-Coated Emulsion Formulation of Docetaxel; Genetaxy; Liposome-encapsulated paclitaxel (LEP); OncoGel; Paciliner Microspheres; PacoExra; Paxoral; PEG-paclitaxel; S-8184; SPS-210C; Taxoprexin; Taxosomes/Dermos; Xytox; Taxoprexin; RPR109881; IXabeplone; Abraxane.

Examples of neurooncologic agents include, without limitation, Antineoplaston A10, Antineoplaston AS-2, Batabulin (T76), Gimatecan, I NO 1001 (Pardex), I rofulven, IXabeplone, CC 8490, Celgene, TP38, Cilenitide; Enzastaurin; Erlotinib (tarceva); Gefitinib (irensa); Vatalanib; AP 12099; Elaproxiral (elaproxy); Motexafin-Gd (xytrin); Temozolomide.

Examples of suitable non-steroidal anti-inflammatory agents include, without limitation, oxicams, such as piroxicam, isoxicam, tenoxicam, sildoxicam, and C514, 304; salicylates, such as aspirin, salicilid, benzylate, triminate, safpyn, solpin, difusilin, and fendosol; acidic derivative, such as dichlofenac, fenofenac, indomethacin, sulindac, tometin, isoxepe, fuorenac, tizopain, ziodmetacin, acetamin, fentizacin, zopepin, clindacin, oxepinac, felbini, and ketorolac; fenamates, such as melenamic, meclofenamic, flufenamic, niflumic, and tolkenamic acids; proionic acid derivatives, such as ibuprofen, naproxen, benoxaprofen, flurbiprofen, ketoprofen, fenoprofen, fenbuten, indoprofen, pirprofen, carprofen, oxaprozin, pranoprofen, miroprofen, tioxaprofen, suprofen, alminoprofen, and tiaprofenic; pyrazol, such as phenylbutazone, oxyphenbutazone, feprazone, azapropropone, and trimethazone. Mixtures of these non-steroidal anti-inflammatory agents may also be employed, as well as the dermatologically acceptable salts and esters of these agents. For example, etofenamate, a flufenamic acid derivative, is particularly useful for topical application.

Examples of suitable steroidal anti-inflammatory agents include, without limitation, corticosteroids such as hydrocortisone, hydroxytriamcinolone, alpha-methyl dexamethasone, dexamethasone-phosphate, beclomethasone dipropionate, clobetasol valerate, desonide, desoxymetha- sone, desoxytococsterone acetate, dexamethasone, dichelisone, diflornesone diacetate, diflucortolone valerate, fluadrenolone, flucorzone acetoneide, fludarcortisone, flumethasone valerate, flusinolone acetone; flutocortisone, flutamone acetone; fludrocortisone acetate, hydrocortisone butyrate, methylprednisolone, triamcinolone acetone; cortisone, cortodoxone, flucetitone, fludrocortisone, diflurahzone diacetate, fluradren- nalone, fludrocortisone, diflurahzone diacetate, fluradrena- lone acetone; medrystone, amincinol, acinfaide, betamethasone and the balance of its esters, chloroprednisone, chlorpredni sonate, clocortolone, clocitoline, dichlorisone, diflurprednone, flurandone, flumisole, flumethamone, fluperonol, fluprednisolone, hydrocortisone valerate, hydrocortisone cyclopropylpropionate, hydrocortisone, meprednisone, paramethasone, prednisolone, prednisone, beclometasone diproprionate, triamcinolone, and mixtures thereof.

Examples of suitable antiinflammatory agents include, without limitation, metclidazine and tripeprazone.

Examples of suitable anesthetic agents include, without limitation, lidocaine, bupivacaine, chlorprocaine, dicain, etocaine, mepivacaine, etracaine, dyclone, hyclaine, procaine, cocaine, ketamine, pramoxine and phenol.

Examples of suitable antimicrobial agents, including antibacterial, antifungal, antiprototol and antiviral agents, include, without limitation, beta-lactam drugs, quinolone drugs, ciprofloxacin, norfloxacin, tetracycline, erythromycin, amikacin, triclosan, doxycycline, capreomycin, chlorhexidine, clortetacycline, oxytetacycline, clindamycin, ethambutol, metronidazole, pentamidine, gentamicin, kanamycin, lineomycin, methacycline, methenamine, minocycline, neomycin, netilmicin, streptomycin, tobramycin, and miconazole. Also included are tetracycline hydrochloride, farnesol, erythromycin estolate, erythromycin stearate (salt), amikacin sulfate, doxycycline hydrochloride, chlorhexidine gluconate, chlorhexidine hydrochloride, chlor tetacycline hydrochloride, oxytetacycline hydrochloride, clindamycin hydrochloride, ethambutol hydrochloride, metronidazole hydrochloride, pentamidine hydrochloride, gentamicin sulfate, kanamycin sulfate, lineomycin hydrochloride, methacycline hydrochloride, methenamine hippurate, methenamine mandelate, minocycline hydrochloride, neo mycin sulfate, netilmicin sulfate, paromomycin sulfate, streptomycin sulfate, tobramycin sulfate, miconazole hydrochloride, amanafide hydrochloride, amanafide sulfate, triclosan, octopirox, parachlorometa xenyl, nystatin, tolnafate and clotrimazole and mixtures thereof.

Examples of suitable anti-oxidants include, without limitation, ascorbic acid (vitamin C) and its salts, ascorbyl esters of fatty acids, ascorbic acid derivatives (e.g., magnesium ascorbyl phosphate, sodium ascorbyl phosphate, ascor byl sorbate), tocopherol (vitamin E), tocopherol sorbate, tocopherol acetate, other esters of tocopherol, butylated hydroxy benzoic acids and their salts, 2,5-dihydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (commercially available under the trade name Trolox®), gallic acid and its alkyl
esters, especially propyl gallate, uric acid and its salts and alkyl esters, sorbic acid and its salts, lipoic acid, amines (e.g., N,N-diethylhydroxylamine, amino-8-guanidine), sulphydryl compounds (e.g., glutathione), dihydroxy furanic acid and its salts, lycine pidolate, arginine pidolate, nordihydroguaiaretic acid, bioflavonoids, curcumin, lycine, methionine, proline, superoxide dismutase, silymarin, tea extracts, grape skin/seed extracts, melanin, and rosemary extracts.

[0208] Examples of suitable antidepressants include, without limitation, norepinephrine-reuptake inhibitors ("NRIs"), selective-serotonin-reuptake inhibitors (SSRIs), monoamine-oxidase inhibitors (MAOIs), serotonin-noradrenaline-reuptake inhibitors ("SNRIs"), corticotropin-releasing factor (CRF) antagonists, α-adrenoreceptor antagonists, NK1-receptor antagonists, 5-HT1a-receptor agonist, antagonists, and partial agonists and atypical antidepressants, as well as norepinephrine-reuptake inhibitors such as, but are not limited to anamitryptiline, desmethylandamitryptiline, clomi-primane, doxepin, imipramine, imipramine-oxide, trimiprimane, adnaxol, amitriptylinoxide, amoxapine, desipramine, maprotiline, norpotryptiline, propyptriline, amineptine, butryptiline, demexiptiline, dibenzipin, dimetra-cine, dothiepin, flucinize, iprindole, loperamine, meltracen, metapramine, norclorprimane, nioxipilin, opipram-ine, perlapine, pizotyline, propizapine, quinuprin, troploxpum, duloxetine, etoporidine, fentoxetine, fluvoxetine, fluvoxamine, indalpine, indoxafalone, milnacipran, nefazodone, oxaffazone, paroxetine, prolintane, ritaferin, sartraline, tandospirone, venlafaxine and zimelidine.

[0209] Examples of suitable vitamins include, without limitation, vitamin A and its analogs and derivatives: retinol, retinal, retinyl palmitate, retinoic acid, tretinoin, isotretinoin (known collectively as retinoids), vitamin E (tocopherol and its derivatives), vitamin C (L-ascorbic acid and its esters and other derivatives), vitamin B6 (niacinamide and its derivatives), alpha hydroxy acids (such as glycolic acid, lactic acid, tartaric acid, malic acid, citric acid, etc.) and beta hydroxy acids (such as salicylic acid and the like).

[0210] Examples of suitable antibacterinames include, without limitation, chlorpheniramine, brompheniramine, deschlorpheniramine, tri扑oline, clemastine, diphenhydramine, promethazine, piperezines, piperidines, astemizole, loratadine and terfenadine.

[0211] Examples of suitable hormones include, without limitation, androgeneic compounds and progestin compounds.

[0212] Examples of suitable androgenic compounds include, without limitation, methyltestosterone, androstere- one, androstene acetate, androstene propionate, androstere- one benzoate, androstenediol, androstenediol-3-ace-tate, androstenediol-17-acetate, androstenediol-3-17-diacetate, androstenediol-17-benzoate, androstenedione, androstenedione, androstenediol, dehydroepiandrosterone, sodium dehydroepiandrosterone sulfate, dromostanolone, dromostanolone propionate, ethyleneol, fluoromestone, nandrolone phenpropionate, nandrolone decanoate, nan- drolone furypropionate, nandrolone cyclohexane-propionate, nandrolone benzoate, nandrolone cyclohexanecarboxylic acid, androstenediol-3-17-benzoate, oxandrolone, oxymetholone, stanozolol, testosterone, test- osterone decanoate, 4-dihydrotestosterone, 5α-dihydrotest- osterone, testolactone, 17α-methyl-19-nortestosterone and pharmaceutically acceptable esters and salts thereof, and combinations of any of the foregoing.

[0213] Examples of suitable progestin compounds include, without limitation, desogestrel, dydrogesterone, ethyliodiol diacetate, medroxyprogesterone, levonorgestrel, medroxy-progesterone acetate, hydroxyprogesterone caproate, nore-thindrone, norethindrone acetate, norethynodrel, alyllestrenol, 19-nortesterone, lynoestrenol, quingestanol acetate, medrogestone, norgestrenione, dimethisterone, ethisterone, cyproterone acetate, chlormadinone acetate, megestrol acetate, norgestimate, norgestrel, comedegestone, gestodene, nomegestrol acetate, progestosterone, 5α-pregn-3β,20α-diol sulfate, 5α-pregn-3β-ol-20-one, 16,5α-pregn-3β-ol-20-one, 4-pregn-20α-ol-3-ol-20-sulfate, acetoxypregnenedolone, anagestrone acetate, cyproterone, ethisterone, fluorogestone acetate, gestodene, hydroxyprogesterone acetate, hydroxyethylprogestosterone, hydroxyethyl progesterone acetate, 3-ketodesogestrel, megestrol, melengestrol acetate, norethisterone and mixtures thereof.

[0214] Also contemplated are agents which are presently being used in animal as well as phase I, II and III of clinical studies for convection-based therapy of brain tumors and other brain pathologies. Representative examples include, without limitation, agents known as: IL13-PE38QQR, TP-38, IL.4(38-37)-PE38 KDEL, cpiIL4.PE, IL-4 cytoxin, IL-4 Pseudomomas exotoxin (NM-3001), TransMED, TCRM107, HSV-1- tk gene-bearing liposomal vector, II-12, IS-FV-IL.12, SP-DPT, DAB39EGF, gemcitabine, carboplatin, glucocere- brosidase, AV2-TK, L IPO-HSV-1-k gene transfer system, TRAIL, BD-EGF and IMC-C225 (as molecular targeting agents for boron neutron capture therapy), interleukin (Il)-1beta and interferon (IFN)-gamma, HAMLET, 6-hydroxy- dopamine, targeted chimera cytoxic proteins, AraC, AAV-2, topotecan, double-stranded RNA-dependent protein kinase PKR, and AP 12009. These agents are presently in animal as well as phase I, II and III of clinical studies for convection-based therapy of brain tumors and other brain pathologies.


[0216] An advantageous approach for introducing a poly- nucleotide, and which is in accordance with preferred embodiments of the present invention, is by using a viral vector. Viral vectors offer several advantages, including higher efficiency of transformation and targeting to, and propagation in, specific cell types. Viral vectors can also be
modified with specific receptors or ligands to alter target specificity through specific cell receptors, such as neuronal cell receptors (Kaspar, B. K. et al. 2002, Mol Ther 5, 50-56).

[0217] Retroviral vectors represent one class of vectors suitable for use with the present invention. Defective retroviruses are routinely used in the transfer of genes into mammalian cells (for a review, see Miller, A. D., 1990, Blood 76, 271). Portions of the retroviral genome can be removed to render the retrovirus replication machinery defective, and the replication-deficient retrovirus can then be packaged into virions, which can be used to infect target cells through the use of a helper virus while employing standard techniques. Protocols for producing recombinant retroviruses and for infecting cells with viruses in vitro or in vivo can be found in, for example, Ausubel et al., 1994, Retroviruses have been used to introduce a variety of genes into many different cell types, including neuronal cells, epithelial cells, endothelial cells, lymphocytes, myoblasts, hepatocytes and bone marrow cells.

[0218] Another suitable expression vector may be an adenovirus vector. The adenovirus is an extensively studied and routinely used gene transfer vector. Key advantages of an adenovirus vector include relatively high transduction efficiency of dividing and quiescent cells, natural tropism to a wide range of epithelial tissues, and easy production of high titers (Russell, W. C., 2000, J Gen Virol 81, 57-63). The adenovirus DNA is transported to the nucleus, but does not integrate thereinto. Thus the risk of mutagenesis with adenoviral vectors is minimized, while short-term expression is particularly suitable for treating cancer cells. Adenoviral vectors used in experimental cancer treatments are described by Seth et al., “Adenoviral vectors for cancer gene therapy,” 1999, pp. 103-120, P. Seth, ed., Adenoviruses: Basic Biology to Gene Therapy, Landes, Austin, Tex.).

[0219] A suitable viral expression vector may also be a chimeric adenovirus/retrovirus vector combining retroviral and adenoviral components. Such vectors may be more efficient than traditional expression vectors for transducing tumor cells (Pan et al. 2002, Cancer Letts 184, 179-188).

[0220] A specific example of a suitable viral vector for introducing and expressing the polynucleotide sequence of the present invention in an individual is the adenovirus-derived vector Ad-TK. This vector expresses a herpes virus thymidine kinase (TK) gene for either positive or negative selection and includes an expression cassette for the desired recombinant sequences. This vector may be used to infect cells that have an adenovirus receptor, which includes most cancers of epithelial origin (Sandmaier et al., 2000, Hum Gene Ther 11, 2197-2205).

[0221] Features that limit expression to particular cell types can also be included on a vector construct. Such features include, for example, promoter and regulatory elements that are specific for the desired target cell type. Secretion signals generally contain a short sequence (7-20 residues) of hydrophobic amino acids. Secretion signals are widely available and are well known in the art (see, e.g., von Heijne, 1985, J Mol Biol 184, 99-105 and Lej et al., 1987, J Bacteriol 169, 4379).

[0222] Although a recombinant vector does not have to be administered locally, local administration can provide a quicker and more effective treatment. Following convective infusion, the viral vectors will circulate until they recognize host cells with appropriate target specificity for infection.

[0223] For any preparation used in the methods of various exemplary embodiments of the present invention, the dosage or the therapeutically effective amount of the therapeutic agents can be initially estimated from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titers. Such information can be used to more accurately determine effective doses in humans.

[0224] Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration, and dosage can be chosen by the individual physician in view of the patient’s condition (see, e.g., Fingl, E. et al. 1975, “The Pharmacological Basis of Therapeutics,” Ch. 1, p. 1.)

[0225] Additional therapeutic agents which can be used include neurotrophic factors (e.g., NGF, BDNF, NT-3, NT-4, NT-5, GDNF), neuroprotective factors (e.g., Eldepryl, Selegeline), endogenous angiogenic-inhibitors (e.g., 2-Methoxyestradiol, Angiostatic corticosteroids, angiostatin, endostatin, Antithrombin III, Arresten, Canstatin, Cartilage-derived inhibitor, CD59 complement fragment, Fibronectin fragments, Growth-related oncogene-beta, Heparin hexasaccharide fragment, Heparinuses, Human chorionic gonadotropin, Interferon-alpha, beta, gamma, Interferon-inducible protein, Interleukin-12, Kringle-5, Metalloproteinase inhibitors, Metallopondin, Amino-terminal fragment of platelet factor-4, Pigment epithelium-derived factor, Placental ribonuclease inhibitor, Plasminogen activator inhibitor, Platelet factor-4, Prolactin M, 16,000 fragment, Proliferin-related protein, Proteamine, angiogenic inhibitors (e.g., Avastin, Topotecan, Thalidomide, CC-5013, Carboxamidotrizole, EMD-121974, COL-3, Marimastat, Prinomastat, Pegylated-interferon alpha-2b, Celecoxib, LY317615), Anti-metabolites (e.g., Methotrexate, cisplatin, DM-phenacyonate hydrochloride, geranylgeranylacetone, nabumetone), Anti-angiogenic factors (e.g., endostatin, avastin), angiogenic factors of the VEGF family (e.g., VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-D1, VEGF-D5, VEGF-145), angiogenic factors of the angiotopoietin family (e.g., Ang-1, Ang-2), angiogenic factors of the FGF family (e.g., alphaFGF, bFGF, FGF-3 to FGF-23), angiogenic factors of the PDGF family (e.g., PDGF-AA, PDGF-BB, and PDGF-AB), angiogenic factors of the TGF family (e.g., TGF-alpha, TGF-beta) or any angiogenic factor (including, without limitation EGF, TNF-alpha, HGF, EPHrins, Integrin alphaV, Integrin beta, Interleukin-12, Interferon-alpha, Interferon-beta, Interferon-gamma, Nitric oxide, Thrombin, Midkine). Also contemplated are IVIG, IGG, HTS, ADCON-L gel, stimulated macrophages, Dihydro-beta-Agarofuran Sesquiterpenes and resiniferatoxin which is known to be useful treatment of painful disorders such as trigeminal neuralgia.

[0226] The composition of the present invention can be used in treatment of many cases of neurodegenerative diseases, ischemic diseases, psychiatric conditions, intractable pain, blockers of angiogenesis, promotion of angiogenesis, inborn errors of metabolism, trauma, infectious diseases, autoimmune diseases. Representative examples include, without limitation epilepsy in which case the composition preferably comprises muscimol which a currently tested for such use, Parkinson, in which case the composition preferably comprises at least one of: muscimol, Sinemet (L-Dopa),
Artane, Amantadine, GDNF, pramipexole (mirapex), ropinirole (reprp), entacapone (comtan), (permax), ropinirole and mitochondrial nutrients; multiple sclerosis, in which case the composition preferably comprises at least one of: interferon, betaseron, copeoxone, avone, mitoxanthrone, resip, methotrexate and cyclophosphamide; dementia, in which case the composition preferably comprises donepezil (aricept); Alzheimer, in which case the composition preferably comprises at least one of: Namenda, flurbiprofen analogues, EGb 761, cholinesterase inhibitors, Statins, rivastigmine, donepezil, Cholinesterase inhibitors, mitochondrial nutrients, memantine, ChEFl, Cloquindol, Juliflorine, gingko biloba, estrogen, non-steroidal anti-inflammatory drugs, vitamin E, and cholinesterase inhibitors; adrenoleukodystrophy, in which case the composition preferably comprises at least one of: long chain fatty acids, mono-saturated fatty acids, and a combination of oleic and erucic acids (also known as “Lorenzo’s oil”); Stroke, in which case the composition preferably comprises at least one of: TPA, calcium channel blockers, aspirin, ticlid, dipyriramid, coumadin, ruphep, aggrexa, plaiv, low molecular weight heparin, heparin, ace-inhibitors, antiplatelet agents, clopidogrel, ginsenoside Rh1 (1) (a ginseng saponin), CDP-choline liposomes, MMP inhibitors, AMPA Antagonist ZK 200775, MC-1, Encapsulated vascular endothelial growth factor-secreting cell grafts, Atenolol, metoprolol, paracetamol (acetaminophen) and dipyriramol; head trauma, in which case the composition preferably comprises at least one of: ca-channel blockers, tricycles, clonidine, benzimidazole derivatives, Ziconotide, netilmicin, olfoxacin, erythropoietin, Thymosin-beta4, melatonin and Zinc); diseases in the prostate, in which case the composition preferably comprises at least one of: anti-hormonal agents, ooxazole, Silybin, silymarin, histone deacetylase inhibitor LAQ824, Rhodamine-123, docextaxel, prednisone, mitoxantrone, 2-Methoxyestradiol, Pamidronate, 1,24(S)-dihydroxyvitamin D2, OXG-011 (a 2’-methoxyethyl antisense oligonucleotide to clusterin), gefitinib, ribvirvin, cyclophamine, celecoxib, SU6668 (anti-angiogenic drug), inositol hexaphosphate, uracil, tefagur, Testosterone substitution, soy isoflavone genistein and amiloe; diseases in the pancreas, in which case the composition preferably comprises at least one of Silybin, silymarin, alpha-hederin, thymoquinone, Nigella sativa, S 21403 (mitigilane), stovastatin (Sortis), ineretins GIP and GLP-1, Sevelamer Hydrochloride, Thiazolidinediones, Nateglinide, quinone oxidoreductase (NQO1), mycopehonic acid, tacrolimus, NADPH, thioredoxin and gluteroxidin.

It is expected that during the life of this patent many relevant MRI contrast agents will be developed and the scope of the term MRI contrast agent is intended to include all such new technologies a priori.

Additional objects, advantages and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMAPLES

Reference is now made to the following examples, which together with the above descriptions illustrate the invention in a non limiting fashion.

Example 1

In accordance with various exemplary embodiments of the present invention, rats subjected to CED treatment in the brain were imaged by MRI. The MR images were analyzed and used to monitor the convection of the administrated agent and to determine the cytotoxic response of the treated tissue.

Methods

Experiment Design

Solutions containing combinations of Cremophor, Taxol®, Carboplatin, Ethanol, sucrose and human serum albumin (HSA) in different concentrations were mixed with Gd-DTPA (1:70). The resultant solution was infused into the striatum of normal Sprague-Dawley (SD) rats (males, 250-300 grams). T1-weighted MR images were acquired immediately post treatment to assess the extent of convection. Additionally, T2-weighted and diffusion-weighted MR images were acquired 24 hours post treatment to assess the response of the tissue and its correlation to the extent of convection. Some rats were monitored by MRI for an additional period of several days to demonstrate the subsequent formation of necrosis following the earlier changes observed on T2-weighted and diffusion-weighted MRI.

CED Procedure

Under full anesthesia, a midline scalp incision was made to identify the bregma. A 1 mm burr hole was made in the right region of the skull, 3 mm anterior and 2 mm lateral to the bregma. A 33-gauge needle attached to a 1000 μl syringe (Gastight; Hamilton) was placed stereotactically 5.5 mm deep into the striatum. The infusion was performed using a BASI syringe pump at a rate of 1 μl/min for a duration ranging from 17 to 90 min.

In order to verify that T1-weighted MR images, acquired immediately post treatment, represent the true distribution of the agent in the tissue, 11 rats were treated by CED with a composition containing 0.1% Evans Blue and Gd-DTPA (1:70) for 30 minutes. 7 out of these 11 rats were treated with a composition having higher viscosity, obtained by adding sucrose at a concentration of 17%. T1-weighted MRI were acquired immediately post treatment, after which the brains were extracted and fixed in Formalin. The relation between the volume of the immediate Gd-DTPA enhancement and the volume of the Evans Blue distribution in the fixed rat brain was studied.

In order to study the correlation between the extent of CED as depicted in the immediate T1-weighted images and the cytotoxic tissue response as depicted in the later diffusion-weighted MR images, 9 SD rats were treated by CED of Taxol® (0.54 mg/ml). In order to obtain a wide range in volumes of distribution, the infusion times were varied between 17 to 60 minutes. 13 rats were treated with Carboplatin (4 mg/ml, 17 minutes infusion). In order to obtain a wide range in volumes of distributions, in 6 out of those, the composition viscosity was increased by adding sucrose to the solution prior to infusion (Carboplatin 4 mg/ml, sucrose concentration ~12%).

In order to study the tissue response to non-toxic agents, 13 rats were treated with several concentrations of human serum albumin (0.2%, 1.0% and 2.5%) and 12 rats with sucrose (5% and 20%) for a duration of 90 minutes.

Imaging

Immediate monitoring of CED formation and extent was performed using T1-weighted images obtained by a Gen-
eral Electric™ 0.5 T interventional MRI machine [Signa SP/i (special proceeding/interventional)] with 1x operating system and gradients intensity of up to 1 Gauss/cm. The T1-weighted images were acquired with: 256x128 matrix, 12x9 cm² field of view (FOV), repetition time (TR) of 400 ms, echo time (TE) of 12 ms and 3 mm slices with no gap.

**[0237]** Early assessment of tissue response was performed using diffusion-weighted and T2-weighted MR images. The diffusion-weighted images were obtained by acquiring conventional T2-weighted images with the addition of diffusion-weighting gradients that filter out the signal from high mobility water molecules and sensitizes the MR images to molecular diffusion/mobility [Stejskal et al. supra]. Regions of accumulated liquids or severe necrosis appear dark in diffusion-weighted MRI, while regions of slow water accumulation, such as in the case of intracellular water accumulation or inflammation, appear brighter.

**[0238]** The diffusion-weighted and T2-weighted MR images were obtained by a General Electric™ 3.0 T MRI machine with 10.4M 1x operating system, gradients intensity of up to 4.3 Gauss/cm and line scan diffusion-weighted imaging [Gudbjartsson et al., “Line scan diffusion imaging”, Magn. Reson. Med. 36:509-519, 1996] acquisition software package. Specially designed animal volume coils of 5 cm diameter were used for data acquisition.

**[0239]** The T2-weighted fast spin echo (FSE) MR images were acquired with: 256x128 matrix, FOV=12x9 cm², TR=3000 ms, TE=90 ms, and 2 mm slices with 0.5 mm gap.

**[0240]** The line-scan diffusion-weighted MR images were acquired with: 256x128 matrix, FOV=12x9 cm², TR=5440 ms, TE=142 ms and 2 mm slices with 0.5 mm gap. The diffusion-weighted MR images were acquired at diffusion weighting factor b of 5 sec/mm² and 1000 sec/mm².

**Image Processing**

**[0241]** The volume (in cm³) of the composition distribution was calculated from the T1-weighted MR images acquired immediately post CED treatment. Region-of-interests (ROIs) were defined over the entire enhancing region in each slice (excluding the ventricles). The number of pixels in the ROIs was counted and multiplying by the volume of a single pixel. Equation 1 was used to calculate ADC maps of the diffusion-weighted MR images.

**Results**

**Convection Monitoring**

**[0242]** The extent of convection formation was reflected by T1-weighted MR images acquired immediately post infusion with infusates mixed with Gd-DTPA. Poor convection was characterized by significant backflow along the catheter and into the ventricles, depicted in the images as significant enhancement in the ventricles and little/no enhancement in the striatum. Efficient convection presented significant spread into the striatum with minimal backflow into the ventricles.

**[0243]** Reference is now made to FIGS. 6A-H which demonstrates the ability of the present embodiments to monitor convection during direct convective interstitial infusion.

**[0244]** FIGS. 6A-B are T1 and T2-weighted axial MR images of normal rat brain. FIGS. 6C-E show T1-weighted MRI acquired immediately post CED treatment with infusates containing Gd-DTPA and Evans Blue. FIGS. 6C-E demonstrate different convective efficiency as depicted by MRI: poor (FIG. 6C), moderate (FIG. 6D) and efficient (FIG. 6E) convection.

**[0245]** FIGS. 6F-H show fixated brain samples of the same rats of FIGS. 6C-E, demonstrating similar distributions of the Evans Blue dye in the tissue.

**[0246]** A highly significant correlation (R²=0.95, p<0.0001, Pearson correlation) was found between the volume of the immediate Gd-DTPA enhancement (see FIGS. 6C-E) and the volume of the Evans Blue distribution (FIGS. 6F-H). The correlation between the two volumes of distribution was found to be highly significant.

**Determination of Cytotoxic Response**

**[0247]** Efficient convection with toxic infusates were followed by significant enhancement in T2-weighted and diffusion-weighted MR images acquired 24 hours post treatment. Efficient prolonged (90 minutes) CED of non-toxic infusates, such as sucrose and human serum albumin solutions, was followed by minor enhancement on T2-weighted and diffusion-weighted MR images, at the site of the catheter tip. When convection was not achieved, including with toxic infusates, no changes were detected on the later T2-weighted and diffusion-weighted MR images.

**[0248]** FIGS. 7A-C are diffusion-weighted MR images acquired for cases of efficient convection, 24 hours post treatment. Shown in FIGS. 7A-C are diffusion-weighted MR images for rats treated with Taxol® (FIG. 7A), Cremophor (FIG. 7B) and Carboplatin (FIG. 7C). As shown in FIGS. 7A-C efficient convection is followed by significant enhancement in diffusion-weighted MR images.

**[0249]** FIG. 8A is a T1-weighted MR image, acquired immediately following the CED of Taxol® mixed with Gd-DTPA. The bright area corresponds to Taxol® distribution in the rat striatum. As shown in FIG. 8A the Taxol® was efficiently distributed over a large area of the striatum.

**[0250]** FIGS. 8B-C are T2-weighted and diffusion-weighted MR images of the same rat as FIG. 8A, acquired 24 hours post treatment. The contrast enhancement corresponds to the cytotoxic response of the tissue to the distributed Taxol®.

**[0251]** FIG. 8D is a low-power magnification of an en block-resected lesion taken from the same rat brain as FIGS. 8A-C. The brain was extracted immediately after the last MR scan. Severe damage covering most of the striatum is seen with widespread necrosis and early gliotic changes. As shown in FIG. 8D the damaged region correlates with the bright regions of the T2-weighted and diffusion-weighted MR images of FIG. 8B-C, thus demonstrating the ability of determining the cytotoxic response of the tissue by MRI.

**[0252]** FIGS. 9A1-A3 are T1-weighted MR images acquired immediately post treatment with Gd-DTPA (catheter position marked in black). FIGS. 9B1-D3 are T1-weighted (FIGS. 9B1-B3), T2-weighted (FIGS. 9C1-C3) and diffusion-weighted (FIGS. 9D1-D3) MR images acquired 24 hours post treatment. FIGS. 9A1, 9B1, 9C1 and 9D1 demonstrate poor convection with 0.2% human serum albumin, no toxicity; FIGS. 9A2, 9B2, 9C2 and 9D2 demonstrate efficient convection with 17% sucrose, minimal toxicity; and FIGS. 9A3, 9B3, 9C3 and 9D3 demonstrate efficient convection with Taxol®, severe tissue toxicity.

**[0253]** As shown in the T1-weighted MR images, there is no residual Gd-DTPA in the brain. As shown in the
T2-weighted and diffusion-weighted MR images, various tissue cytotoxic responses were observed.

Reference is now made to FIGS. 10A-C, which demonstrate the relation between the volume of the Gd-DTPA enhancement and the volume of cytotoxic tissue response as studied for the 10 Taxol® treated rats and 13 Carboplatin treated rats. The Gd-DTPA enhancement was determined from T1-weighted MRI taken immediately post treatment and the cytotoxic tissue response was determined from diffusion-weighted MR images taken later, as further detailed above (see Methods).

FIG. 10A is T1-weighted MR image acquired immediately post treatment of one rat with the admixture of Taxol® and Gd-DTPA; FIG. 10B is diffusion-weighted MR image of the same rat acquired 24 post treatment; and FIG. 10C is a correlation graph, showing, on the absissa, the enhancement volume in cm³ as calculated for 10 rats treated with CED of Taxol® from the MR images similar to that of FIG. 10B, and on the ordinate the enhancement volume in cm³ as calculated from images of the same 10 rats similar to that of FIG. 10A. Significant correlation was found between the two enhancement volumes (Taxol®: R²=0.75, p<0.004; Carboplatin: R²=0.67, p<0.002, Pearson correlation), demonstrating the ability of the present embodiments to monitor the convection by T1-weighted MRI and determine the cytotoxic response by diffusion-weighted MRI.

Discussion

In the present examples the application of MRI for monitoring convection as well as determining cytotoxic response of cells to CED was demonstrated. CED formation and extent was monitored by T1-weighted MRI and cytotoxic tissue response was determined by T2-weighted and diffusion-weighted MRI.

Gd-DTPA has been previously used as a surrogate marker for CED distribution, by chemically binding or entrapping it to macromolecules or other particles such as liposomes and co-infusing the labeled particles with the original infusate (Saito et al., “Distribution of liposomes into brain and rat brain tumor models by convection-enhanced delivery monitored with magnetic resonance imaging”, Cancer Research, 1: 64(7):2572-9, 2004; Mamot et al., “Extensive distribution of liposomes in rodent brains and brain tumors following convection-enhanced delivery”, J Neurooncol 68(1):1-9, 2004; Nguyen et al., “Convecive distribution of macromolecules in the primate brain demonstrated using computerized tomography and magnetic resonance imaging”, J Neurosurg, 98(3):584-90, 2003; and Lonser et al., “Successful and safe perfusion of the primate brainstem: in vivo MRI of macromolecular distribution during infusion”, J Neurosurg, 97:905-913, 2002). Gd-DTPA has also been used to monitor the extent of CED with saline [Weber et al., abstract RA-24, Eighth Annual Scientific Meeting of the Society for Neuro-Oncology, Keystone, Colo., November 2003, SNO 2003.]

Attempts have also been made to perform real-time in vivo CT imaging of convection using small radioactive imaging tracer [Croteau et al., J Neurosurg 102:90-97, 2005]. However, although the use of CT for real-time monitoring of CED is problematic because (i) CT has a relatively low anatomical resolution compared to MRI, and (ii) CT can not be used for multiple imaging sessions due to accumulation of radiation.

The present embodiments successfully monitor therapeutic agents convection within tissues by co-formulating (e.g., mixing) the therapeutic agents with an MRI contrast agent (e.g., Gd-DTPA) prior to infusion, and imaging the tissue, contemporaneously, immediately post infusion or at a later time. Such procedure can provide real-time assessment of formation efficiency and extent of CED. The significant correlation between the extent of CED, as depicted by the immediate T1-weighted images, and the distribution of the Evans Blue dye (similar molecular weight to Taxol) in the fixed rat brain samples, as well as the significant correlation with the later cytotoxic tissue responses, as depicted by the DWMR images, in the cases of Taxol and Carboplatin, confirms the validity of the present embodiments.

Since Gd-DTPA is a small particle, it is carried along with the convective wave of the therapeutic agents. Short clearance time of Gd-DTPA from the tissue was observed in 3 cases. For these cases, sequential T1-weighted MRI images showed clearance of significant percentage of the Gd-DTPA within 2-4 hours post infusion (data not shown). It is assumed that for larger molecules the clearance time is longer.

Early assessment of tissue cytotoxic response to treatment is essential, especially in the case of brain tumors, where both efficient treatments of pathological regions as well as sparing of normal tissue are critical. Accurate non-invasive treatment monitoring can enable treatment adjustment in real-time thus optimizing treatment outcome on a patient by patient basis.

The non-specific cytotoxic tissue response, depicted as enhancing regions on T2-weighted and diffusion-weighted MR images, is consistent with previous clinical findings obtained by the present inventors [Mardor et al; Lidar et al., supra], where data of 15 patients with recurrent GBM treated by CED of Taxol® were presented. This clinical data showed that early changes in the diffusion-weighted MR images were followed by later tumor necrosis. Patients who did not present early changes on the diffusion-weighted MR images had no later radiological responses to treatment. On the other hand, Mardor et al. and Lidar et al. fail to identify the reason for the early changes in the diffusion-weighted MR images. In particular, Mardor et al. and Lidar et al. fail to correlate enhancement in diffusion-weighted MR images with tissue cytotoxic response. The finding presented in the present example and obtained in accordance with various exemplary embodiments of the present invention, successfully provide clarification as to the reason of the early changes observed by Mardor et al. and Lidar et al. Specifically, the present embodiments allow the identification of enhancement regions in diffusion-weighted MR images with the tissue cytotoxic response to the Taxol®. Moreover, the explanation for no response in some of the patients in Lidar’s clinical trial is the lack (or low efficiency) convection and consequently small distribution volume of Taxol® in the tissue.

Efficient prolonged (90 minutes) CED of non-toxic infusion, was followed by minor enhancement on T2-weighted and diffusion-weighted MR images, at the site of the catheter tip. This minor damage to the tissue can be explained by the large pressure gradient caused by the continuous infusion. This finding is consistent with the calculations by Chen et al. [Chen et al., “intraparenchymal drug delivery via positive-pressure infusion: experimental and modeling studies of poroelasticity in brain phantom gels”,]
IEEE Transactions on Biomedical Engineering, 49(2):85-96, 2002, which showed that for long infusion times the pore fraction increases at short radial distances from the catheter tip.

[0265] The present animal study demonstrates the ability of MRI to determine cytotoxic tissue response. Diffusion-weighted MRI is somewhat advantageous over T2-weighted MRI since it provides additional information (see FIGS. 8A-H). Diffusion-weighted MRI is particularly advantageous for the detection of cytotoxic tissue response in brain tumors, because most brain pathologies are accompanied by vasogenic edema which appears bright in T2-weighted MR images. The heterogeneous appearance of pre-treatment pathology, in addition to vasogenic brain edema, may screen the T2 changes resulting from the treatment. In diffusion-weighted MRI, on the other hand, most of the signal from vasogenic edema is filtered out, thus making this technique more effective than T2-weighted MRI, at least for the detection of cytotoxic tissue response in brain tumors.

Example 2

[0266] In accordance with various exemplary embodiments of the present invention, high viscosity infusates were used for distributing Iron Oxide (IO) nano particles in rat brain by CED.

Methods

[0267] The impact of enhanced viscosity on CED of large particles was tested for 10 nano-particles (20 nm dry size, 70 nm wet size).

Experiment 1

[0268] A low viscosity solution consisting of IO biocompatible and biodegradable particles suspended in saline and a high viscosity solution, consisting of the same concentration of IO particles, suspended in a saline solution containing HSA were infused into the rat striatum. The infusion was performed at a rate of 1 μl/min for duration of 30 minutes. Immediate monitoring of CED formation and extent was performed using gradient echo MR images.

Experiment 2

[0269] IO particles, as in Experiment 1 were covered with Dextran to increase viscosity and infused into rat brain. The infusion was performed at a rate of 4 μl/min for duration of 15 minutes. 24 rats were scanned immediately post treatment by MRI and 5 were followed by MRI for 4 weeks. One rat was treated with concentration of 0.2 mg/ml for 7.5 minutes at a rate of 8 μl/min. Two rats were treated with a high concentration infusate (2 mg/ml), and subsequently their brains were harvested to assess IO distribution by direct visualization. Three rats were treated with a low 0.05 mg/ml in a 20% sucrose solution to increase viscosity. Eight rats were treated by IO particles labeled by Rhodamine B Amine, and subsequently the brains were harvested and evaluated by MRI and spectral imaging.

Results

Experiment 1

[0270] FIGS. 11A-B show Sagittal slices of gradient echo MRI acquired immediately post infusion with the low viscosity IO infusate (2 rats, FIG. 11A) and the high viscosity IO infusate (2 rats, FIG. 11B). The presence of IO particles is shown in the gradient echo MR images as a dark region near the catheter tip.

[0271] As shown in FIGS. 11A-B, although the two solutions (low and high viscosity) had an identical number of IO particles, the CED of the low viscosity IO infusate resulted in a dark region which is significantly smaller (about 1-2 mm in diameter) compared to the artifact caused by using the viscous infusate (4 mm diameter). It is therefore demonstrated that the efficacy and extent of conversion strongly depends on the viscosity of the infused composition.

Experiment 2

[0272] FIGS. 12A-D show gradient-echo MR images taken immediately post treatment with a 0.2 mg/ml infusate at 8 μl/min over 7.5 minutes (FIG. 12A); gradient-echo MR images taken immediately post treatment with a 0.2 mg/ml infusate at 4 μl/min over 15 minutes (FIG. 12B); direct visualization of a brain harvested immediately post treatment with the high concentration infusate (FIG. 12C); and spectral image taken immediately post treatment by the fluorescent-labeled IO.

[0273] As shown in FIG. 12A the treatment with 0.2 mg/ml infusate at 8 μl/min over 7.5 minutes, resulted in poor CED which was observed as backflow of the IO particles into the ventricles. As shown in FIGS. 12B-D, the other treatments resulted in efficient CED observed as homogenous distribution of IO in the striatum.

[0274] FIGS. 13A-D and FIGS. 14A-D show gradient-echo MR images taken for rat Nos. 1 (FIGS. 13A-D) and 2 (FIGS. 14A-D), immediately (FIGS. 13A and 14A), 3 days (FIGS. 13B and 14B), 6 days (FIGS. 13C and 14C) and 27 days (FIGS. 13D and 14D) post the CED treatment with a 0.2 mg/ml infusate at 4 μl/min over 15 minutes.

[0275] The maximal distribution reached was 31.4 mm². The long-term follow-up showed near linear clearance of the particles, reaching 20-40% of the original distribution after 4 weeks. No toxicity was detected. In the fluorescent and the direct IO visualization studies the distribution areas were 7.8 and 8.2 mm², respectively (see FIGS. 12C-D).

[0276] FIGS. 15A-C show gradient-echo MR images taken immediately post treatment with low concentration IO (0.05 mg/ml) in the 20% sucrose solution at infusion rates of 1 μl/min over 30 minutes (FIGS. 15A-B), and 2 μl/min over 15 minutes (FIG. 15D). As shown, the sucrose in the low concentration infusate caused aggregation of the IO particles resulting in no penetration of the IO into the tissue.

[0277] The present example demonstrates that the high viscosity of the infusates, according to the teaching of the present embodiments, enables a rapid (15 mints infusion time) and efficient distribution of large particles via CED. The clearance time of the large particles was significantly longer than that of smaller particles. Additionally, the present example demonstrates the use of MR imaging for real time direct depiction of IO distribution.

Example 3

[0278] In accordance with various exemplary embodiments of the present invention, high viscosity infusates were used for distributing high viscosity toxic infusates in mice thigh.

Methods

[0279] Five balb-c mice, bearing large (over 2 cm in diameter) C26 colon cancer tumors in the thigh, were treated with
CED of high viscosity toxic infusates. Three mice were treated with high concentration Paclitaxel (1.2 mg/ml) at infusion rate of 4 ml/min for 60 minutes. Two mice were treated with a high concentration combination of Ethanol and Cremaphore (150 μl Ethanol and 150 μl Cremaphore diluted with Saline to 1 ml) at infusion rate of 4 μl/min for 105 minutes.

Infusates were mixed with Gd (1:70) prior to treatment. MR images were acquired immediately post treatment to assess the distribution of the drug within the tumor. The mice treated with Paclitaxel were resuscitated 24 hours post treatment to assess tissue response.

Results

[0281] FIGS. 16A-H: show an example of a large C26 tumor induced in the thigh of a baboon mouse treated by CED of high concentration Paclitaxel: FIGS. 16A-B are T2 MR images of the tumor prior to treatment; FIGS. 16C-D are T1 MR images taken immediately post treatment; FIGS. 16E-G are T2 MR images taken 24 hours post treatment; and FIG. 16H is an image of the treated mouse. The Gd-enhanced shown in FIGS. 16C-D depicts the efficient distribution of the drug in the tumor during the CED treatment. The maximal distribution diameter was about 1.4 cm. As shown in the 24 hours follow-up (FIG. 16E-G), the high Paclitaxel concentration and effective distribution has caused complete necrosis of the tumor tissue in regions treated by CED within 24 hours or less.

[0283] Similar results were obtained for the other mouse the Ethanol/Cremaphore combination.

[0284] FIGS. 17A-M show an example of a large C26 tumor induced in the thigh of a baboon mouse treated by CED of high concentration Paclitaxel: FIGS. 17A-D are T2 MR images of the tumor prior to treatment; FIG. 17E-H are T1 MR images taken immediately post treatment; FIG. 17I-L are T2 MR images taken 24 hours post treatment; and FIG. 17M is an image of the treated mouse. As shown in FIG. 17E-H, a considerable, nearly complete backflow of the drug was observed, revealing inefficient CED. As shown in the 24 hours follow-up there was no significant change in the tumor indicating that the treatment was not effective.

Example 4

[0286] In accordance with various exemplary embodiments of the present invention, rats were treated with high viscosity infusates containing blue bovine serum albumin and Gd-DTPA by CED.

Methods

[0287] Ten rats were treated by CED with an infusate containing 1% blue bovine serum albumin (40 KD) and Gd-DTPA (1:70) at infusion rate of 4 μl/min for 20 minutes. Four out of the ten rats were treated with a higher viscosity infusate obtained by adding human serum albumin at a concentration of 2%. T1-weighted MRI were acquired immediately post treatment, after which the brains were harvested and fixed in Formalin.

Results

[0288] FIGS. 18A-B through FIGS. 22A-B are the T1-weighted MR images acquired immediately post treatment (A) and respective optical images of the tissue samples (B) of five different rats. The correlation between the area of Gd distribution, as depicted in the immediate T1 MR images, and the area of distribution as depicted in the tissue samples was found to be highly significant (r^2=0.92, p<0.0001, Pearson correlation). The significant correlation expresses the ability of the present embodiments to monitor CED extent for larger molecules.

Example 5

[0289] Following is an exemplified protocol for formulating a composition for CED. Different solvents were used for dissolving Taxol so as to test for non-specific neurotoxicity. The different solvents were prepared from different combinations of drugs selected from the group consisting of Peg 8000, Tween 80, DMSO, Cremaphore, Ethanol, Saline and Gd-DTPA were used in CED.

[0290] For each solvent, immediate monitoring of CED formation and extent was performed using T1-weighted images, and assessment CED toxicity was performed using T2-weighted MR images.

[0291] FIGS. 23A-H are T1-weighted MR images acquired immediately post treatment (FIGS. 23A-D) and respective T2-weighted MR images acquired 24 hours post treatment (FIGS. 23E-H). The MR images demonstrate moderate toxicity (FIGS. 23A and 23E, FIGS. 23B and 23F), minor toxicity (FIGS. 23C and 23G) and no toxicity (FIGS. 23D and 23H).

[0292] More generally, the protocol can include the following method steps which are illustrated in the flowchart diagram of FIG. 24.

[0293] Hence, the method for formulating a composition for CED begins at step 60 and continues to step 62 in which direct convective interstitial infusion catheter is placed in one or more animals. The method continues to step 64 in which a composition, mixed with an MRI agent is pressured through the catheter. The method continues to step 66 in which the tissue contacting the catheter and surrounding tissues are imaged by MRI, during or immediately post treatment.

[0294] The method continues to step 67 in which the convection extent is assessed from the MRI images. Assessment of CED formation and extent can then be performed, for example, by obtaining T1-weighted MR images immediately post treatment, and calculating the distribution volume of the composition. The calculation can be performed by defining regions-of-interests over the entire enhanced region of the images, and multiplying the number of pixels in the regions-of-interests by the volume which corresponds to a single pixel.

[0295] The method then proceeds to decision step 68 in which the methods determines whether or not the convection extent is satisfactory (e.g., whether there is low or no backflow). If the convection extent is not satisfactory, the method continues to step 70 in which the viscosity of the composition is increased as further detailed hereinabove. From step 70 the method loops back to step 64.

[0296] If, on the other hand the convection extent is satisfactory, the method proceeds to step 71 in which the in which the tissue contacting the catheter and surrounding tissues are imaged by MRI, preferably T2-weighted and/or diffusion-weighted MRI, immediately after or several hours (e.g., 24 hours) post treatment. The method then continues to step 72 in which non-specific cytotoxic tissue response is assessed based on the images acquired in step 71. The method then proceeds to decision step 74 in which the methods determines
whether or not the non-specific toxicity is low. If the non-specific toxicity is not low, the method continues to step 76 in which the composition is reformulated by manipulating the drug solvents or other physical, chemical, biological characteristics of the composition to decrease the cytotoxicity. From step 76 the method loops back to step 64, in which the above method steps are executed for an additional group of animals.

[0297] If the non-specific toxicity is low (e.g., it cannot be reduced by a reasonable number, say 5-10 iterations or more) the method ends at step 80.

[0298] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

[0299] Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

1-75. (canceled)

76. A method of determining a response of a tissue to a destructive treatment, the method comprising providing magnetic resonance images of the tissue, and using said magnetic resonance images for determining the response of the tissue to the destructive treatment.

77. The method of claim 76, wherein said destructive treatment comprises a convection-enhanced drug delivery.

78. The method of claim 76, further comprising imaging the tissue.

79. The method of claim 78, wherein said imaging is effected by diffusion-weighted magnetic resonance imaging.

80. The method of claim 78, wherein said imaging is effected by T2-weighted magnetic resonance imaging.

81. The method of claim 78, further comprising administering an MRI contrast agent to the tissue prior to said imaging of the tissue.

82. A method of generating a catheter acceptance map for convection-enhanced drug delivery into an organ, the method comprising providing at least one image of the organ, analyzing said at least one image so as to identify different regions corresponding to different catheter acceptance levels for efficient convection, and marking said regions so as to allow distinction between said different regions, thereby generating the catheter acceptance map for convection-enhanced drug delivery into the organ.

83. A catheter acceptance map of an organ, comprising at least one image of the organ having distinctly marked regions, each corresponding to a different catheter acceptance levels for efficient convection via convection-enhanced drug delivery.

84. The method of claim 82, wherein said at least one image comprises at least one of: a magnetic resonance image, a single photon emission computed tomography image, a positron emission tomography and diffusion tensor magnetic resonance image.

85. A method of monitoring convection during direct convective interstitial infusion, the method comprising: providing a pharmaceutical composition which comprises a therapeutic agent and an MRI contrast agent, the pharmaceutical composition is in liquid form; and pressuring said pharmaceutical composition through at least one direct convective interstitial infusion catheter into an interstitial volume of a tissue; imaging a region containing the tissue by MRI thereby providing at least one image of said region; and using said at least one image for monitoring the convection of said therapeutic agent within the tissue.

86. The method of claim 85, wherein said using said at least one image for monitoring the convection comprises comparing the images with baseline images.

87. The method of claim 85, wherein said using said at least one image for monitoring the convection comprises correlating intensity level the images with level of presence of said therapeutic agent.

88. The method of claim 85, wherein said using said at least one image for monitoring the convection comprises correlating intensity level the images with a concentration of said therapeutic agent.

89. The method of claim 85, wherein said using said at least one image for monitoring the convection comprises calculating distribution volume of said pharmaceutical composition.

90. The method of claim 85, wherein said using said at least one image for monitoring the convection comprises calculating expansion rate of said pharmaceutical composition.

91. The method of claim 85, wherein said using said at least one image for monitoring the convection comprises determining a direction of expansion of said pharmaceutical composition.

92. The method of claim 85, wherein said using said at least one image for monitoring the convection comprises detection backflow of said pharmaceutical composition along said at least one direct convective interstitial infusion catheter.

93. The method of claim 85, wherein said using said at least one image for monitoring the convection comprises detection leakage of said pharmaceutical composition into low resistance paths.

94. A system for determining a response of a tissue to a destructive treatment, the system comprising an MRI apparatus for providing magnetic resonance images of the tissue, and an image processing unit for processing said magnetic resonance images so as to determine the response of the tissue to the destructive treatment.

95. The method of claim 76, wherein said magnetic resonance images comprise diffusion-weighted magnetic resonance images.

96. The method of claim 76, wherein said magnetic resonance images comprise T2-weighted magnetic resonance images.

97. The method of claim 95, wherein said determining the response of the tissue comprises calculating apparent diffusion coefficient (ADC) of the tissue.

98. The system of claim 94, further comprising a destructive treatment apparatus for performing a destructive treatment.
99. The method of claim 76, wherein said destructive treat-
ment comprises a convection-enhanced drug delivery.

100. The method of claim 76, wherein said destructive treat-
ment comprises a systemic chemotherapy.

101. The method of claim 76, wherein the destructive treat-
ment comprises destruction by gene therapy or gene-related
therapy.

102. The method of claim 76, wherein the destructive treat-
ment comprises a thermal treatment.

103. The method of claim 76, wherein the destructive treat-
ment comprises cryotherapy.

104. The method of claim 76, wherein the destructive treat-
ment comprises ablation.

105. The method of claim 76, wherein the destructive treat-
ment comprises heat ablation.

106. The method of claim 76, wherein the destructive treat-
ment comprises ultrasound ablation.

107. The method of claim 76, wherein the destructive treat-
ment comprises RF ablation.

108. The method of claim 76, wherein the destructive treat-
ment comprises a photodynamic therapy.

109. The method of claim 76, wherein the destructive treat-
ment comprises an electrosurgical treatment.

110. A system for monitoring convection during direct
convective interstitial infusion, the system comprising:

an MRI apparatus for providing magnetic resonance
images of a region containing the tissue;

at least one direct convective interstitial infusion catheter
for delivering at least one pharmaceutical composition
having a therapeutic agent dissolved or dispersed in a
liquid pharmaceutical carrier and at least one MRI con-
trast agent; and

an image processing unit for processing said magnetic
resonance images so as to monitor the convection of said
therapeutic agent within the tissue.

111. The system of claim 110, wherein said image process-
ing unit is operable to compare said images with baseline
images.

112. The system of claim 110, wherein said image process-
ing unit is operable to correlate intensity level of said images
with level of presence of said therapeutic agent.

113. The system of claim 110, wherein said image process-
ing unit is operable to correlate intensity level of said images
with a concentration of said therapeutic agent.

114. The system of claim 110, wherein said image process-
ing unit is operable to calculate distribution volume of said
pharmaceutical composition.

115. The system of claim 110, further comprising said
pharmaceutical composition.

116. The method of claim 85, wherein said at least one MRI
contrast agent has a T1 shortening effect.

117. The method of claim 85, wherein said at least one MRI
contrast agent has a T2 shortening effect.

118. The method of claim 116, wherein said at least one
MRI contrast agent comprises a diethylenetriamine pentaacetic
acid (DTPA).

119. The method of claim 116, wherein said paramagnetic
metal comprises Gadolinium (Gd).

120. The method of claim 116, wherein said at least one
MRI contrast agent comprises Gadolinium-diethylenetri-
amine pentaacetic acid (Gd-DTPA).

121. The method of claim 116, wherein said images com-
prise T1-weighted magnetic resonance images.