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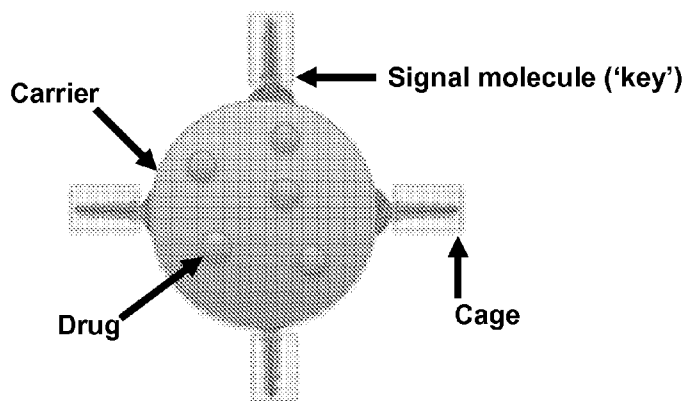


Figure 1

(57) Abstract: Disclosed are methods, kits and compositions pertaining to controllably delivering an agent to a cell. In various aspects and embodiments the present technology may involve a composition that includes an agent and a targeting moiety wherein the targeting moiety is configured to switch from an inactive configuration to an active configuration responsive to an external stimulus. In some embodiments, the targeting moiety in the active, but not inactive, configuration allows for delivery of the agent to the cell.



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COMPOSITIONS AND METHODS FOR CONTROLLABLY DELIVERING AN AGENT

TECHNICAL FIELD

[0001] This disclosure relates generally to methods, kits and compositions pertaining to controllably delivering an agent to a cell.

BACKGROUND

[0002] The following description is provided to assist the understanding of the reader. None of the information provided or references cited is admitted to be prior art.

[0003] The ability to control delivery and action of agents such as bioactive agents is desirable in a number of various types of settings. US Patent No 6,017,758 discloses the use of a di-methoxy nitro phenyl ethyl group (DMNPE) as a photolabile caging group on an isolated nucleic acid that reversibly prevents expression of the nucleic acid. A review of compositions and methods pertaining to caging and uncaging various types of agents with photolabile groups is provided in GCR Ellis-Davies, Nature Methods 4:619-628 (2007). US Patent Application Publication No. 2006/0128814 at discloses delivering a nanoparticle to a targeted site, exposing the nanoparticle to light that cleaves photolabile bonds in the monomer units of the nanoparticle and, thus, releasing a biological material to the targeted site.

SUMMARY

[0004] The present technology disclosed herein is based at least in part on the discovery of methods and compositions that may be used for controllably delivering an agent to a cell.

[0005] In one aspect, provided are compositions for controllably delivering an agent to a cell. The compositions may include a nanocapsule that includes an agent and a targeting moiety. The targeting moiety may be configured to switch from an inactive configuration to an active configuration responsive to an external stimulus. In certain embodiments the targeting moiety is capable of binding to the cell when in the active configuration and is not capable of, or is less capable of binding to the cell when in the inactive configuration. In

certain embodiments the targeting moiety, when in the active configuration, is capable of binding a protein on the surface of the cell. In certain embodiments, the protein on the surface of the cell is a receptor, for example the receptor may be a receptor for a hormone, a receptor for a growth factor, a receptor for a neurotransmitter, a receptor for a blood protein, or the like. In certain illustrative embodiments, the protein on the surface of the cell is a transferrin receptor.

[0006] In one aspect, provided are kits that include a composition in accordance with the present technology. In some embodiments the kit further includes instructions for use, for example the instructions may include instructions to administer the composition (for example any composition such as described herein) to a cell or subject, and to apply the external stimulus to the composition.

[0007] In one aspect, provided are methods of manufacturing compositions and nanocapsules for controllably delivering an agent to a cell. For example, the methods may include forming a nanocapsule comprising one or more agents and a targeting molecule in an inactive configuration; wherein the targeting molecule switches from the inactive configuration to an active configuration in response to an external stimulus.

[0008] In one aspect, provided are methods for controllably delivering an agent to a cell. The method may include administering a composition such as described herein to the cell. In some embodiments, the methods involve administering an agent to a cell or population of cells, for example a cell or population of cells present in a subject, a cell or population of cells *in vivo*, or a cell or population of cells *in vitro*. In some embodiments the method may include administering to a cell and/or subject a composition such as described herein that includes a nanocapsule containing the agent. The nanocapsule may include a surface moiety in an inactive configuration, wherein the targeting moiety in the inactive configuration does not bind to the cell. After administering the composition to the cell or subject, the method may further involve exposing the nanocapsule to light, wherein the light changes the targeting moiety from the inactive configuration to an active configuration, and wherein the targeting moiety in the active configuration binds to the surface of the cell or the population of cells in the subject and the agent enters the cell by endocytosis.

[0009] In some illustrative embodiments of the compositions and methods of the present technology, the targeting moiety includes a peptide, for example a peptide that includes the

amino acid sequence, SEQ ID NO. 1 (HAIYPRH). In certain embodiments, the targeting moiety in the inactive configuration includes a group such as a photolabile group. In some embodiments the targeting moiety the inactive configuration includes a group such as a photolabile group and the targeting moiety in the active configuration does not include the group. The photolabile group may, for example, be a di-methoxy nitro phenyl ethyl (DMNPE) group. In some embodiments the targeting moiety is a peptide and a DMNPE group is bound to the carboxylic acid terminus of the targeting moiety when the targeting moiety is in the inactive configuration.

[0010] In certain embodiments the external stimulus may be light. In some such embodiments where the external stimulus is light, the targeting moiety in the inactive configuration includes a photolabile group and the targeting moiety in the active configuration does not have the photolabile group. In some illustrative embodiments, the external stimulus may be light having a wavelength between 300-450 nm. In some illustrative embodiments the stimulus may be chemical, heat, a magnetic field or an electric field.

[0011] In some embodiments of the nanocapsules of the present technology and the methods of using the nanocapsules such as described herein, the nanocapsule and/or agent is taken into the cell by endocytosis after the targeting moiety in the active configuration binds to the surface of the cell.

[0012] In some embodiments of the methods and compositions of present technology, the agent is one or more selected from the group consisting of a biologically active molecule, a drug, a cytokine, a nucleic acid molecule, a DNA molecule, RNA molecule, a molecule capable of exhibiting RNA interference (RNAi) activity, a molecule capable of expressing a gene, or the like.

[0013] In accordance with the present technology, the composition and/or nanocapsule may be administered to a subject by one or more of intravenous, intramuscular, oral, intraperitoneal administration, or any other route of administration suitable to contact the composition and/or nanocapsule with the cell. In some embodiments where the composition is administered to a subject, the composition is administered to the subject at a site in the subject that is different than the site that includes the population of cells. In some embodiments of the method, the nanocapsule is exposed to the external stimulus (*e.g.*, light)

at least 5 minutes after the administration to the cell or subject; or at least 30 minutes after the administration to the cell or subject; or at least 1 hour after the administration to the cell or subject; or at least 3 hours after the administration to the cell or subject.

[0014] The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative aspects, embodiments, and features described above, further aspects, embodiments, and features will become apparent by reference to the following drawings and the detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0015] Figure 1 shows a cartoon of the components in an illustrative example of a composition of the present technology.

[0016] Figure 2 shows a cartoon illustrative of the an example of the present technology, where the signaling peptide or targeting moiety (represented as a key) attached to the carrier is uncaged by light. Prior to this, the cage prevented the key from interacting with the receptor (represented as a lock). After uncaging, the peptide activated the receptor, allowing the entire capsule inside the cell via endocytosis.

[0017] Figure 3 illustrates an illustrative embodiment of a reaction to form a DMNPE derivative (above) from a carboxylic acid end of a peptide that yields a 'caged peptide' that can act in a targeting moiety in an inactive configuration. The ester that is formed by the reaction changes the chemical and physical properties of the peptide, preventing it from activating its target receptor. When light of the appropriate frequency is shined on it, the DMNPE ester is cleaved, allowing the peptide to exhibit its intended functionality (such as targeted endocytosis).

DETAILED DESCRIPTION

[0018] In the following detailed description, reference may be made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting.

Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented here.

[0019] Unless otherwise stated, the singular forms “a,” “an,” and “the” as used herein include plural reference.

Controllable Delivery

[0020] In many situations it is beneficial (and often required) to deliver many drugs directly into cells, rather than waiting for them to diffuse from the extracellular space into the cell interior. One class of drug that may require this treatment is RNAs, which have a very short extracellular half-life. Endocytosis of drugs may be accomplished by physical means (such as careful mixing retinoic-acid loaded particles with cells), or by chemical means, such as incorporating them into a liposome designed to merge with the cell. Generally these methods may require delivering the agent at the beginning of the tissue culture process; whereas it may in some circumstances be beneficial to induce endocytosis later during development, and preferentially ‘on demand’ by the user. Accordingly, in certain aspects and embodiments the present technology provides compositions and methods for controlling endocytosis of agents by a cell.

[0021] Accordingly, the present technology provides, *inter alia*, methods and compositions for endocytosis of a nanocapsule or other drug/carrier combination triggered by an external stimulus to allow for controlled delivery and endocytosis. For example, a nanocapsule containing a cytokine, RNA, or other bioactive molecule may be decorated with a targeting moiety that may in certain circumstances have the capability to recognize or bind to a cell. In certain embodiments the targeting moiety may have an active and inactive configuration; for example, in the inactive configuration the targeting moiety may be “caged,” *i.e.*, it may have or include a group such as a photolabile group that blocks or prevents its ability to bind to the cell. “Uncaging” this moiety, or removing the group such as a photolabile group from the targeting moiety, enables the freed targeting moiety to bind to the surface of a cell and/or trigger endocytosis of the capsule and/or agent (*i.e.*, uncaging the targeting moiety switches the targeting moiety from the inactive to active configuration). This creates a mechanism to provide agents to the cell only when required or desired, and may have applications in controlling the differentiation, growth and maturation of cells and tissues.

[0022] Thus, in certain aspects and embodiments the present technology provides methods and compositions for controllably delivering agents to a cell at a prescribed time in which the agent is presented in a composition in a caged form where it is unrecognized or less-recognized by the cell (*i.e.*, in an inactive configuration) until uncaged by an external stimulus, and applying an external stimulus to uncage a targeting moiety (for example to remove a group from the targeting moiety or to change the configuration of the targeting moiety). In certain embodiments the composition in an inactive or caged configuration has an affinity for the cell (or an ability to be recognized by the cell) that is less than that of the composition in an active or uncaged configuration by a factor of at least 2; or at least 3; or at least 4; or at least 5; or at least 6; or at least 7; or at least 8; or at least 9; or at least 10. In some embodiments, the uncaged or active configuration of the targeting moiety acts as a trigger for endocytosis of the composition or agent into the cell. Thus, in many embodiments the agent only enters the cell after the application of the external stimulus.

[0023] For example, the present technology may include an agent encapsulated in a nano-sized matrix (for example, but not limited to nanocapsules, liposomes, vesicles, and the like), for example as represented on Figure 1, with a caged targeting moiety (referred to as a “signaling molecule” or “key” on Figure 1) present on the surface of the matrix. The agent/carrier may be added to a cell, a subject, tissue culture or the like, and in certain embodiments the agent is inert (or less effective) while outside the cellular environment. If the carrier is a controlled-release matrix, the agent may be slowly released as the matrix degrades, but would be ineffective because it is outside the cell. Once the targeting moiety or signaling molecule is triggered with an external stimulus (for example, light), the signaling molecule (targeting moiety) is uncaged and thus in the active configuration, and can interact with a receptor to trigger endocytosis, for example as illustrated in the cartoon of Figure 2. Thus, the carrier is brought inside the cell. Once the carrier is inside the cell, the agent is slowly released into the cellular environment where it is efficacious. Non-limiting examples of agents that can be efficacious inside a cellular environment but may be inert or less effective while outside the cellular environment include, RNA, DNA, siRNA, plasmids, expression constructs, transcription factors and the like.

Applications and Agents for Delivery

[0024] The methods and compositions may be applicable in any circumstance where it is desirable to have the ability to control the time and area an agent is delivered. In certain

embodiments, the agent is delivered to a cell or population of cells. In some illustrative embodiments a cell or population of cells may be present in a subject, or a tissue, or part of a tissue. In some embodiments a cell or population of cells may be present in a subject, tissue or part of a tissue *in vivo*. In certain embodiments a cell or population of cells may be *in vitro*, for example in a cell culture or tissue culture environment. The term “subject” as used herein refers to plant, animal, mammal, human, patient, bacteria, virus, fungus, or anything that includes a collection or population of cells. The term “population of cells” as used herein refers to any collection of cells, including a collection of cells present in an *in vitro* culture, in a subject, in a tissue, in a part of a tissue, or the like.

[0025] In certain embodiments, the compositions and methods of the present technology may be applied such as to allow for endocytosis and/or delivery of an agent only at the time and area exposed to an stimulus, for example an external stimulus. In some embodiments, the stimulus is applied to a localized area of the subject or cultured cells such as to allow endocytosis only in a specified area. In one illustrative embodiment the stimulus may be light, and the light may be applied locally using a rasterizing laser and/or a photomask to control the area in which the light is applied. For example, in one illustrative embodiment the stimulus (such as light) may be applied to a subject only in an area in the subject where there is a tumor, allowing the agent to specifically target the tumor cells with less effects on a part of the subject not believed to have a tumor. In another illustrative embodiment, the stimulus (such as light) may be selectively applied to cells in a cell culture dish such that only cells in a certain region are exposed to the stimulus and cells outside that region are not. This embodiment will allow a comparison of the cells exposed to the stimulus (and thus affected by the agent) with those that are not using a single culture of cells in a single dish. In some embodiments, a composition of the present technology is exposed to the external stimulus (e.g., light) at least 5 minutes after the administration to the cell or subject; or at least 30 minutes after the administration to the cell or subject; or at least 1 hour after the administration to the cell or subject; or at least 3 hours after the administration to the cell or subject; or at least 5 hours after the administration to the cell or subject; or at least 10 hours after the administration to the cell or subject; or at least 12 hours after the administration to the cell or subject; or at least 24 hours after the administration to the cell or subject; or at least 2 days after the administration to the cell or subject; or at least 4 days after the administration to the cell or subject such as to temporally control the delivery and/or endocytosis of the agent. In some embodiments, a composition of the present technology is exposed to the

external stimulus (e.g., light) approximately 5 minutes after the administration to the cell or subject; or approximately 30 minutes after the administration to the cell or subject; or approximately 1 hour after the administration to the cell or subject; or approximately 3 hours after the administration to the cell or subject; or approximately 5 hours after the administration to the cell or subject; or approximately 10 hours after the administration to the cell or subject; or approximately 12 hours after the administration to the cell or subject; or approximately 24 hours after the administration to the cell or subject; or approximately 2 days after the administration to the cell or subject; or approximately 4 days after the administration to the cell or subject such as to temporally control the delivery and/or endocytosis of the agent. In some embodiments, a composition of the present technology is exposed to the external stimulus (e.g., light) between 5 and 10 minutes after the administration to the cell or subject; or between 15 and 45 minutes after the administration to the cell or subject; or between 30 minutes and 1 hour after the administration to the cell or subject; or between 2 and 4 hours after the administration to the cell or subject; or between 4 and 8 hours after the administration to the cell or subject; or between 8 and 12 hours after the administration to the cell or subject; or between 12 and 16 hours after the administration to the cell or subject; or between 20 and 28 hours after the administration to the cell or subject; or between 1 and 3 days after the administration to the cell or subject; or between 3 and 5 days after the administration to the cell or subject such as to temporally control the delivery and/or endocytosis of the agent.

[0026] In certain embodiments, the compositions and methods of the present technology may be used to controllably deliver an agent to a cell, for example in *in vitro* cell or tissue culture conditions. In such embodiments the compositions may be in a suitable form or buffer for *in vitro* cell culture procedures.

[0027] For example, the delivery of an appropriate cytokine to a cell at an appropriate time is controlled by homeostasis in living organisms, but must be artificially modulated in tissue engineering. An example of the importance of this has been demonstrated in studies of vascularization, where release of the cytokine VEGF is necessary to encourage vascular growth, whereas the later release of PDGF is required for cell maturation. If the release of each growth factor is appropriately timed, the resulting blood vessels are healthier than if the factors are used simultaneously (see for example, TP Richardson *et al.*, Nature Biotechnology 19:1029-1034 (2007), hereby incorporated by reference in its entirety). Accordingly, the

present technology may, in certain embodiments, be used to for time controlled delivery of agents such as cytokines in regulating cell development.

[0028] It has also been demonstrated that siRNAs can be used to impact the fate of differentiating stem cells. As a non-limiting example, using an siRNA to inhibit the production of the TAZ protein by a particular stem cell line, the cells were much more likely to differentiate to adipocytes than osteoblasts than untreated cells (see for example Jeong-Ho Hong, *et al.*, Science, 309:1074-1078, (2005), hereby incorporated by reference in its entirety). Accordingly, in certain embodiments the present technology may be used to controllably deliver siRNA to cells.

[0029] In some embodiments, the present technology is useful in tissue engineering applications. For example, compositions or nanoparticles of the present technology may be added to an engineered tissue and deliver an agent only at the time and area exposed to an external stimulus. In some embodiments, the external stimulus is applied to localized area of the tissue for delivery of the agent only in a localized area. In some illustrative embodiments, the stimulus may be light that is applied locally only to a portion of the tissue using a rasterizing laser and/or a photomask to control the area in which the light is applied. In other embodiments, the external stimulus is applied to the entire tissue to promote delivery of an agent at a particular time. In a certain such illustrative embodiment, a composition of the present technology that is in the inactive configuration may be embedded within or bound to a tissue engineering scaffold; the cells and tissues can be allowed to grow and develop on the scaffold with no or minimal effect or influence of an agent within the composition; and once the cells or tissues have achieved a certain level of growth or development the stimulus may be applied thus activating composition as well as the effects of the agent. Several examples of controlling the fate of mature tissue through genetic engineering have also been attempted. In certain tissue engineering applications nucleic acids may be used to induce (or inhibit) the synthesis of the appropriate growth factors (see for example Yamamoto and Tabata, Adv Drug Deliv Rev. 58:535-5 (2006) hereby incorporated by reference in its entirety). For example, the present technology may be used to deliver DNA encoding for PDGF or another protein or factor, rather than delivering the factor itself. Similar approaches can be used to control tissue development: for example, TGF- β is known to increase the production of extracellular matrix (ECM), and down-regulating TGF- β also down-regulated ECM

production. This approach may be useful to harmonize ECM production to the rate of scaffold degradation, for example.

[0030] In some embodiments, the compositions of the present technology (*e.g.*, nanocapsules such as described herein) are administered to a subject such as a mammal or a human. For example, the agent may be a biologically active compound (such as a drug, hormone, growth factor (cytokine), RNA, nucleic acid) in which the ability to regulate endocytosis and/or delivery of an agent in the subject is advantageous. In such applications, the composition (for example a nanocapsule) such as described herein may be in a form suitable for administration to an animal or human.

[0031] Administration to the subject may be in any way suitable, for example, oral administration, intravenous administration, intramuscular administration, intraperitoneal administration, administration by suppositories, inhalation administration, and the like. The dosage to be administered depends to a large extent on the condition and size of the subject being treated as well as the frequency of treatment and the route of administration. As such, provided herein is a pharmaceutical product which may include a composition and/or a nanocapsule, as described herein may be a pharmaceutically acceptable injectable or administrable carrier and suitable for introduction to a tissue or cells *in vivo*, for example in a pharmaceutically acceptable form for administration to a human and/or animal approved by an appropriate government agency. In some embodiments, the external stimulation may be applied at a localized area of the subject that may be the same or different to the area of that the composition is applied. For example in some embodiments the external stimulus is localized light applied to an area of the subject that is different than the area of administration. In some illustrative embodiments, the composition is administered systemically (such as intravenously) to a subject and the stimulus may selectively be applied only to an area where it is advantageous to have the agent exert its activity; for example, where the agent has anti-tumor or cancer activity the stimulus may be selectively applied to an area of the subject where a tumor (such as a skin tumor) is present or suspected. In some embodiments, in which the composition and stimulus is administered to a subject or cell, the stimulus may be applied in a manner that minimizes harm to the cells or tissues; or to cells or tissues different than those targeted by the stimulus. In embodiments where the stimulus is light this may be done, for example, by modulating the wavelength and/or intensity of the light, and/or using a focused light (*e.g.*, a laser) or photomask such as to achieve sufficient

stimulation to activate the composition in the desired area while minimizing harm to cells or tissues.

[0032] In some embodiments of the methods and compositions of present technology, the agent is one or more selected from the group consisting of a biologically active molecule, a drug, a cytokine, a nucleic acid molecule, a DNA molecule, RNA molecule, a molecule capable of exhibiting RNA interference (RNAi) activity, a molecule capable of expressing a gene, or the like. In certain embodiments, the present technology may be particularly applicable in the delivery and/or controlled endocytosis of agents that are based on RNA or include RNA as such agents can initiate (or prevent) differentiation and other biological functionality in a cell, and may often be difficult to deliver in a timed manner because they degrade immediately in an extracellular environment. In some embodiments, the agent is a protein or a peptide; for example a protein or a peptide that is a growth factor or cytokine.

Targeting Moieties

[0033] A targeting moiety of the present technology may be any moiety that can target a desired cell. The targeting moiety may be configured to switch from an inactive configuration to an active configuration responsive to an external stimulus (for example, the inactive configuration may be a “caged” form and the active configuration may be an “uncaged form”). In certain embodiments the targeting moiety is capable of binding to a cell when in the active configuration and is not capable of, or is less capable of binding to the cell when in the inactive configuration. In certain embodiments the targeting moiety in an inactive or caged configuration has an affinity for the cell (or an ability to be recognized by the cell) that is less than that of the targeting moiety in an active or uncaged configuration by a factor of at least 2; or at least 3; or at least 4; or at least 5; or at least 6; or at least 7; or at least 8; or at least 9; or at least 10. In certain embodiments, the targeting moiety, when in the active configuration, is capable of binding a protein on the surface of the cell. In certain embodiments, the active state targeting moiety (*e.g.*, an uncaged targeting moiety) once bound to the surface of the cell is able to facilitate endocytosis such as to cause the composition, nanocapsule, and/or agent to enter the cell.

[0034] The protein on the surface of a cell to which the active targeting moiety may bind may, in certain embodiments, be a receptor. For example the receptor may be a receptor for a hormone such as, but not limited to, an insulin receptor, a follicle stimulating hormone

receptor, an epidermal growth factor receptor, a growth hormone receptor, a thyroid stimulating hormone receptor, a luteinizing hormone receptor, a thyroid hormone receptor, an erythropoietin receptor, a glucagon receptor, a leptin receptor, a prolactin receptor, a calcitonin receptor, a receptor for a catecholamine hormone, or a melanocyte stimulating hormone receptor; a receptor for a growth factor such as, but not limited to, a VEGF receptor, a TGF- β superfamily member receptor, a nerve growth factor receptor, a platelet derived growth factor receptor, an interferon receptor, or an IGF receptor; a receptor for a neurotransmitter such as, but not limited to, a dopamine receptor, an acetylcholine receptor, a melatonin receptor, a serotonin receptor, or a vasopressin receptor, a receptor for a blood protein such as, but not limited to, a receptor for transferrin, receptor, yolk proteins, IgE, polymeric IgA, maternal IgG, IgG (for example a Fc receptor), a asialoglycoprotein, a low density lipoprotein (LDL), or the like. In some embodiments the targeting moiety is a receptor for a ligand or factor that enters a cell by receptor mediated endocytosis. For example, the targeting moiety may be a receptor for a toxin or lectin, such as but not limited to a receptor for diphtheria toxin, pseudomonas toxin, cholera toxin, ricin, concanavalin A or the like. In some embodiments, the targeting moiety may be a receptor for a virus, such as but not limited to a receptor for rous sarcoma virus, semliki forest virus, vesicular stomatitis virus, adenovirus, influenza virus, RSV virus, or the like.

[0035] In some embodiments, the targeting moiety may be used to specifically target one cell type in the presence of a second cell type by targeting a cell surface receptor that is much more numerous on one cell type than the second cell type. For example, mature osteoblasts have a lower level of transferrin receptor expression than chondrocytes, so use of this pathway would lead to selective delivery to chondrocytes at the osteoblast-chondrocyte interface.

[0036] Thus, in certain illustrative embodiments, the protein on the surface of the cell is a transferrin receptor. The amino acid sequence, SEQ ID NO.1 (HAIYPRH), is an effective small molecule mimic for transferrin. US Patent 6743893; hereby incorporated by reference in its entirety. In embodiments where an active targeting moiety binds a transferrin receptor, the targeting moiety may be a peptide that includes that includes the amino acid sequence, SEQ ID NO.1 (HAIYPRH), that can act as a mimetic for transferrin. In some illustrative embodiments, the protein on the surface of the cell is an LDL receptor and the targeting moiety includes the sequence LRKLRKRLLRDADDLLRKLRKRLLRDADDL (SEQ ID

NO.2) or a dimeric linear tandem repeat of SEQ ID NO.2 (see, for example, Cheryl Dyer, *et al.*, *Journal of Biological Chemistry*, (1991) 266:22,803-22,806; hereby incorporated by reference in its entirety). In some illustrative embodiments the cell surface molecule is a asialoglycoprotein receptor and the targeting moiety is a galactoside ligand-polylysine conjugate with affinity for an asialoglycoprotein receptor, such as but not limited to a compound shown on figure 1 of Cristian Plank, *et.al.*, *Bioconjugate Chem*, (1992) 3:533-539 (hereby incorporated by reference in its entirety).

[0037] In many embodiments the targeting moiety switches from an inactive configuration to an active configuration in response to an external stimulus. In some embodiments, the targeting moiety in the inactive configuration includes a group that blocks the targeting moiety from interacting or binding to the cell (*i.e.*, the inactive targeting moiety is “caged”); and the group is removed or changed in response to the external stimulus such as to switch the targeting moiety to an active configuration and allow binding to the cell (*i.e.*, “uncaging” the targeting moiety). In certain embodiments, the group is a photolabile group that responds to light as an external stimulus. Compositions and methods pertaining to caging and uncaging with photolabile groups that may be used in various embodiments of present technology are disclosed in GCR Ellis-Davies, *Nature Methods* 4:619-628 (2007) and in US Patent No 6,017,758, hereby incorporated by reference in their entirety. Compositions and methods pertaining to photolabile caging groups and chemistries that can be used in various illustrative embodiments in conjunction with the methods and compositions disclosed herein are described in Isabelle Aujard, *et al.*, *Chemistry, European Journal*, (2006) 12:6865-6879, hereby incorporated by reference in its entirety. The photolabile group used in conjunction with the present technology may in some embodiments be a group or moiety selected from the following non-limiting example groups: 1-(ortho-nitrophenyl)-ethyl (NPE); α -carboxy-ortho-nitrobenzyl (CNB); α -carboxy-ortho-nitrobenzyl (CNB); ortho-nitrobenzyl (NB); 7-diethylaminocoumarinyl-4-methyl (DEAC); 6-bromo-7-hydroxycoumarin-4-methyl (Bhc); 4,5-dimethoxy-2-nitrobenzyl (DMNB); a di-methoxy nitro phenyl ethyl (DMNPE) group (*e.g.*, a 1-(4,5-dimethoxy-2-nitrophenyl) group); or 4-methoxy-7-nitroindolinyl (MNI). In certain embodiments, the photolabile group is DMNPE, such as shown in Figure 3.

[0038] In some embodiments in which the targeting moiety is a peptide, a photolabile group or other group is bound or attached to the carboxylic acid terminus of the targeting moiety when the targeting moiety is in the inactive configuration. In some embodiments in which

the targeting moiety is a peptide, the amine side of the targeting moiety is bound to or coupled with a nanocapsule (for example it may be coupled to PPF using chemistries known in the art). In some illustrative embodiments, the linking chemistries described in Isabelle Aujard, *et al.*, Chemistry European Journal, (2006) 12:6865-6879 (hereby incorporated by reference in its entirety) may be used. In some embodiments, the amine side of a targeting moiety is coupled to the nanocapsule by reacting the amine with a fumarate and exposing the molecules to heat. In some embodiments, the amine side of a targeting moiety is coupled to the nanocapsule by reacting the amine with a fumarate and N-bromosuccinimide (NBS) to convert it to a bromamide followed by nucleophilic displacement of the bromide. In certain embodiments in which the targeting moiety is a peptide, a photolabile group or other group is bound or attached to the carboxylic acid terminus of the targeting moiety when the targeting moiety is in the inactive configuration and the amine side of the targeting moiety is bound to or coupled with a nanocapsule.

[0039] The external stimulus may be any stimuli that causes the targeting moiety to switch from an inactive to an active configuration (such as from a caged to an uncaged state). In some illustrative embodiments the stimuli may be chemical, heat, a magnetic field or an electric field.

[0040] In many embodiments the external stimulus may be light. In illustrative embodiments the targeting moiety in the inactive or caged configuration includes a photolabile group. The light may be applied by any suitable light source. For example, the light may be applied using a lamp or flash lamp (for example xenon or mercury arc lamps that may optionally be focused with a parabolic mirror); a laser such as a near-UV laser, a continuous wave AR-KR laser (for example that emits light at 354-363 nm), a pulsed laser (for example a frequency-doubled ruby, pulse-width 35 ns at 347 nm laser or a frequency-tripled Nd-YAG laser; a two photon excitation system (such as a solid-state mode locked Ti:sapphire laser) or the like. In some embodiments, the light may be at a wavelength that is greater than 400 nm, or greater than 500 nm, or greater than 600 nm, or greater than 700 nm. In some illustrative embodiments the wavelength is between 700 and 1000 nm, or between 700 and 800 nm. In some embodiments in which the light needs to travel through cells or tissue, a wavelength between 700 and 1000 nm, or between 700 and 800 nm may be selected because light at this wavelength may be able to penetrate certain tissue without scattering and/or because light at higher wavelengths may be less likely to cause DNA damage. In certain embodiments, the

light may be applied in conjunction with a confocal microscope, a two photon microscope or a laser launcher. The shutters for light sources may be optical or mechanical. In some embodiments, the light source is a femtosecond pulsed laser suitable for use in two-photon applications; and the light wavelength is between 700 and 1000 nm, or between 700 and 800 nm.

[0041] In some embodiments the external stimulus is a magnetic field, electrical field or heat. In certain illustrative embodiments where the external stimulus is a magnetic field, electrical field or heat, the nanocapsule of the present technology is encased in a hydrogel that surrounds the targeting moiety, thus, causing the targeting moiety to be in an inactive configuration. The hydrogel has a melting point slightly above the body temperature or culture temperature where the nanocapsule is administered. The electric field or heat applied as an external stimulus causes the hydrogel to melt, thus exposing the targeting moiety and causing it to switch from the inactive configuration to an active configuration. In certain embodiments, the hydrogel includes superparamagnetic nanoparticles (see, for example, J Dobson, *Gene Therapy*(2006)13:283-287) disposed throughout the hydrogel, the external stimulus is a magnetic field, and the application of the magnetic field external stimulus causes the superparamagnetic nanoparticles to heat, thus melting the hydrogel and exposing the targeting moiety resulting in the targeting moiety switching from an inactive configuration to an active configuration. In certain illustrative embodiments, the magnetic field, electrical field or heat is applied such that the temperature of the hydrogel reaches a temperature that is at least 4° C; or at least 5° C; or at least 6° C; or at least 7° C; or at least 8° C; or at least 9° C; or at least 10° C higher than the culture temperature or body temperature where the matrix is administered. For example, if the culture temperature or body temperature is 37° then the magnetic field, electrical field or heat is applied such that the temperature of the hydrogel reaches at least 41° C; or at least 42° C; or at least 43° C; or at least 44° C; or at least 44° C; or at least 45° C; or at least 46° C; or at least 47 °C. In certain illustrative embodiments, the external stimulus may be applied as described in Derfus, et. al., *Advanced Materials*, 19:3932-3936 (2007), hereby incorporated by reference in its entirety. For example, in certain illustrative embodiments the external stimulus may be an electromagnetic field applied with a 3kW power supply for a period of about 5 minutes.

[0042] In some embodiments the external stimulus is a chemical. In certain illustrative embodiments where the external stimulus is a chemical, the nanocapsule is encased in a

hydrogel that breaks down or dissolves when exposed to the chemical, thus causing the targeting moiety to switch from an inactive to active configuration. In some illustrative embodiments, the hydrogel is, or includes a gelatin that breaks down or dissolves upon exposure to the chemical external stimulus. In certain illustrative embodiments, the chemical is a protease. In some illustrative embodiments the chemical is a papain. In some illustrative embodiments the chemical is a tobacco etch virus protease that specifically cleaves at E-X-X-Y-X-Q-G/S (SEQ ID NO. 3) between Q and G/S and the hydrogel is based on proteins having the E-X-X-Y-X-Q-G/S (SEQ ID NO. 3) sequence. In some embodiments, the hydrogel is or includes nucleic acids and the chemical stimulus is a nuclease.

Nanocapsules

[0043] The term “nanocapsule,” “nanoparticle” or “nanosphere” as used herein refers to particles having a size (*e.g.*, a diameter) between 1 nm and 1,000 nm; or between 1 nm and 600 nm; or between 50 nm and 500 nm; or between 100 nm and 400 nm; or between 150 nm and 350 nm; or between 200 nm and 300 nm. Unless otherwise specified, the terms “nanocapsule,” “nanoparticle” or “nanosphere” as used herein broadly include liposomes, vesicles, emulsions and the like having indicated particle sizes. In certain embodiments, a “nanocapsule composition” as used herein refers to a composition that includes particles wherein at least 30%; or at least 40%; or at least 50%; or at least 60%; or at least 65%; or at least 70%; or at least 75%; or at least 80%; or at least 85%; or at least 87%; or at least 90%; or at least 92%; or at least 95%; or at least 97% of the particles fall within a specified size range, for example wherein the size range is between 1 and 1,000 nm; or between 1 nm and 600 nm; or between 50 nm and 500 nm; or between 100 nm and 400 nm; or between 150 nm and 350 nm; or between 200 nm and 300 nm.

[0044] Nanocapsules as described herein may be made or manufactured using any technique known in the art, including emulsification techniques (including double-emulsification techniques), spray drying techniques, water-in-oil-in-water techniques, syringe extrusion techniques, coaxial air flow methods, mechanical disturbance methods, electrostatic force methods, electrostatic bead generator methods, and/or droplet generator methods. For example, nanoparticles of the present technology may be manufactured using techniques and methods similar to those described in US Patent No. 6,884,432, hereby incorporated by

reference in its entirety. In certain embodiments, nanocapsules of the present technology may be gelatin-based; for example similar to those disclosed in Vandelli, *et al.*, International Journal of Pharmaceutics (2001), 215:175-185. The size and other properties of nanocapsules may be changed by altering various parameters in the production process. Freidberg *et al.*, (2004) 282:1-18 (hereby incorporated by reference in its entirety) provides a review of procedures and compositions for particle manufacture, any of which procedures and compositions may be used in conjunction nanocapsules of the present technology.

[0045] In some embodiments nanocapsules of the present technology may be based on or include a polymer-based matrix. For example nanocapsules may be based on or include poly(propylene fumarate) (PPF). In many embodiments PPF may be advantageous because it contains double bonds that may be reacted with a targeting moiety without otherwise impacting the degradation kinetics of the capsule interior. In some embodiments nanocapsules may be based on or include polylactic acid (PLA), polyglycolic acid (PGA), and/or copoly lactic acid/glycolic acid (PLGA). Each of PLA, PGA and PLGA polymers, like PPF polymers, are biodegradable.

Kits

[0046] The compositions (such as a matrix, as described herein), materials and components described herein may be suited for the preparation of a kit. Thus, the disclosure provides a kit useful for controlled delivery of a compound to a subject or a cell.

[0047] In one embodiment, the methods described herein may be performed by utilizing pre-packaged kits including compositions for controlled delivery (such as a matrix, or a nanocapsule as described herein) and/or materials to administer the controlled delivery compositions and/or materials for applying the external stimulus. The kits may contain instructions for the use of the components included in the kit; for example instructions to administer the composition to a cell or subject, and stimulate the composition with light. In some embodiments, each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package.

[0048] A kit may further include a second container that includes a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and/or dextrose solution. It can further include other materials desirable from a commercial and user

standpoint, including other buffers, diluents, filters, stirrers, needles, syringes, and/or package inserts with indications and/or instructions for use.

[0049] The units dosage ampules or multidose containers, in which the components may be packaged prior to use, and/or may be packaged as a sterile formulation, and the hermetically sealed container is designed to preserve sterility of the formulation until use.

EXAMPLES

[0050] The present compositions, methods and kits, thus generally described, will be understood more readily by reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present methods and kits. The following is a description of the materials and experimental procedures used in the Examples.

Example 1- Generation and Use of Nanocapsules Having Caged Transferrin as a Targeting Moiety.

[0051] DMNPE is coupled to the C-terminus of transferrin using chemistry and techniques known in the art. The amine-terminus of DMNPE-transferrin is coupled to PPF in PPF-based nanocapsules having an active agent that is desired to act on chondrocytes. The nanocapsules are added to a culture that includes osteoblasts and chondrocytes. The nanocapsules are allowed to incubate in the cultures for a period of 3 hours and then are exposed to light having a wavelength of 365 nm. The light exposure causes the disassociation of the DMNPE from the transferrin targeting moiety on the nanocapsules, thus, uncaging the caged transferrin (and, thus, causing the transferrin targeting moiety to switch from an inactive state to an active state). There are no observed effects of the agent on the cells prior to the application of light. Following the application of light, the agent exhibits clear actions in the cell, with the effects on chondrocytes being more prevalent than the effects on osteoblasts due to the greater number of transferrin receptors present on chondrocytes.

Example 2 – Timed Delivery of Cytokines Using Caged Targeting Moiety Nanocapsules.

[0052] Nanocapsules having an uncaged targeting moiety that encapsulate an expression vector having DNA encoding VEGF are prepared. Nanocapsules having a DMNPE-caged targeting moiety that encapsulate an expression vector having DNA encoding PDGF are also prepared. A composition having a mixture of the two types of nanocapsules are then added

to a tissue engineering scaffold with immature tissues. The uncaged nanocapsules having the VEGF expression vector enter the cells of the tissue scaffold by endocytosis and cause expression of VEGF and, in turn, vascular growth in the tissue. Once sufficient vascular growth has occurred, the tissue scaffold with the nanocapsule mixture is exposed to light causing disassociation of the DMNPE from the targeting moiety of the PDGF-nanocapsules, thus causing the targeting moiety to switch from an inactive to an active configuration. The active configuration PDGF-nanocapsules then enter the cells by endocytosis and cause expression of PDGF and, in turn, cell maturation.

Example 3 –Delivery of TAZ siRNA Using Caged Targeting Moiety Nanocapsules.

[0053] Nanocapsules having a DMNPE-caged targeting moiety that encapsulate siRNA against the gene encoding the TAZ protein are prepared. The nanocapsule is added to a culture of differentiating mesenchymal stem cells and allowed to incubate in the culture for one hour. The cell cultures the nanocapsules are exposed to light causing disassociation of the DMNPE from the targeting moiety, thus causing the targeting moiety to switch from an inactive to an active configuration. The active configuration nanocapsules then enter the cells by endocytosis and deliver the siRNA to the inside of the cell. The siRNA causes a down regulation of TAZ protein in the cells and, in turn, cause the cultured stem cells to form more adipocytes than osteoblasts.

REFERENCES

1. Jeffrey A. Engler, Jae Hwy Lee, James F. Collawn, Bryan A. Moore, Receptor mediated uptake of peptides that bind the human transferrin receptor, US Patent 6743893, 2004.
2. GCR Ellis-Davies, “Caged compounds: photorelease technology for control of cellular chemistry and physiology”, Nature Methods 4 (2007) 619-628)
3. Esfandiar Behraves, Kyriacos Zygourakis, Antonios G. Mikos, Adhesion and migration of marrow-derived osteoblasts on injectable in situ crosslinkable poly(propylene fumarate-co-ethylene glycol)-based hydrogels with a covalently linked RGDS peptide, Journal of Biomedical Materials Research Part A, Volume 65A Issue 2, Pages 260 - 270.

4. TP Richardson *et. al.*, "Polymeric system for dual growth factor delivery", Nature Biotechnology 19 (2001) 1029-1034.
5. Jeong-Ho Hong, Eun Sook Hwang, Michael T. McManus, Adam Amsterdam, Yu Tian, Ralitsa Kalmukova, Elisabetta Mueller, Thomas Benjamin, Bruce M. Spiegelman, Phillip A. Sharp, Nancy Hopkins, Michael B. Yaffe, TAZ, a Transcriptional Modulator of Mesenchymal Stem Cell Differentiation, Science, v309, 2005, p1074-1078.
6. Yamamoto M, Tabata Y., Tissue engineering by modulated gene delivery, Adv Drug Deliv Rev. 2006 Jul 7;58(4):535-5.

* * * *

[0054] The present disclosure is not to be limited in terms of the particular embodiments described in this application. Many modifications and variations can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the disclosure, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the appended claims. The present disclosure is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled. It is to be understood that this disclosure is not limited to particular methods, reagents, compounds compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0055] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0056] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper

third, *etc.* As will also be understood by one skilled in the art all language such as “up to,” “at least,” “greater than,” “less than,” and the like include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 particles refers to groups having 1, 2, or 3 particles. Similarly, a group having 1-5 particles refers to groups having 1, 2, 3, 4, or 5 particles, and so forth. As used herein, the term “about” means in quantitative terms, plus or minus 10%.

[0057] While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

CLAIMS

What is claimed is:

1. A composition for controllably delivering an agent to a cell, comprising:
a nanocapsule comprising one or more agents and a targeting moiety,
wherein the targeting moiety is configured to switch from an inactive configuration to an active configuration responsive to an external stimulus.
2. The composition of claim 1, wherein the targeting moiety is capable of binding to a cell when the targeting moiety is in an active configuration and is not capable of binding to the cell when the targeting moiety is in the inactive configuration.
3. The composition of claim 2, wherein the targeting moiety in the active configuration is capable of binding to a protein present on the surface of the cell.
4. The composition of claim 3, wherein the protein present on the surface of the cell is a transferrin receptor.
5. The composition of claim 1, wherein the targeting moiety is capable of initiating endocytosis of the nanocapsule into a cell when the targeting moiety is in an active configuration and is not capable of initiating endocytosis of the nanocapsule into the cell when the targeting moiety is in the inactive configuration.
6. The composition of any of the preceding claims, wherein the targeting moiety is a peptide.
7. The composition of any of the preceding claims, wherein the targeting moiety is a peptide comprising the amino acid sequence, SEQ ID NO.1 (HAIYPRH).
8. The composition of any of the preceding claims, wherein the targeting moiety in the inactive configuration comprises a photolabile group.
9. The composition of claim 8, wherein the photolabile group is a di-methoxy nitro phenyl ethyl (DMNPE) group.

10. The composition of claim 9, wherein the targeting moiety is a peptide and wherein the DMNPE group is bound to the carboxylic acid terminus of the targeting moiety when the targeting moiety is in the inactive configuration.
11. The composition of any of the preceding claims, wherein the external stimulus is light.
12. The composition of any of the preceding claims, wherein the external stimulus is light and wherein the targeting moiety in the inactive configuration comprises a photolabile group and the targeting moiety in the active configuration does not have a photolabile group.
13. The composition of any of the preceding claims, wherein the external stimulus is light having a wavelength between 300-450 nm.
14. The composition of any of claims 1 to 13, wherein the nanocapsule is taken into the cell by endocytosis after the targeting moiety in the active configuration binds to the surface of the cell.
15. The composition of any of the preceding claims, wherein the agent is one or more selected from the group consisting of a biologically active molecule, a drug, a cytokine or an RNA molecule.
16. A kit comprising a composition of any of the preceding claims.
17. The kit of claim 16, further comprising instructions for use.
18. The kit of claim 17, wherein the instructions include instructions to administer the composition to a cell or subject, and to apply the external stimulus to the composition.
19. A method of controllably delivering an agent to a cell, comprising:
applying the external stimulus to the composition of any of the preceding claims.
20. The method of claim 19, wherein the method comprises contacting the composition with a cell in a subject.
21. The method of claim 20, wherein the agent is delivered to the cell before application of the external stimulus.

22. A method of controllably delivering an agent to a population of cells in a subject, comprising:

administering to the subject a composition comprising a nanocapsule containing the agent, wherein the nanocapsule comprises a surface moiety in an inactive configuration and wherein the targeting moiety in the inactive configuration does not bind to the cell, and

exposing the nanocapsule to light, wherein the light changes the targeting moiety from the inactive configuration to an active configuration, wherein the targeting moiety in the active configuration binds to the surface of the cells of the population of cells in the subject and the agent enters the cell by endocytosis.

23. The method of claim 22, wherein the composition is administered by one or more of intravenous, intramuscular, oral, or intraperitoneal administration.

24. The method of any of claims 22 to 23, wherein the composition is administered to the subject at a site in the subject that is different than the site that includes the population of cells.

25. The method of any of claims 22 to 24, wherein the nanocapsules are exposed to light at least 5 minutes after the administration to the subject.

26. The method of any of claims 22 to 24, wherein the nanocapsules are exposed to light at least 30 minutes after the administration to the subject.

27. The method of any of claims 22 to 24, wherein the nanocapsules are exposed to light at least one hour after the administration to the subject.

28. The method of any of claims 22 to 24, wherein the nanocapsules are exposed to light at least 3 hours after the administration to the subject.

29. The method of any of claims 22 to 28, wherein the targeting moiety in the active configuration is capable of binding to a protein present on the surface of the cell.

30. The method of claim 29, wherein the protein present on the surface of the cell is a transferrin receptor.

31. The method of any of claims 22 to 30, wherein the targeting moiety is a peptide.

32. The method of claim 31, wherein the targeting moiety is a peptide comprising the sequence SEQ ID NO:1 (HAIYPRH).
33. The method of any of claims 22 to 32, wherein the targeting moiety in the inactive configuration comprises a photolabile group.
34. The method of claim 33, wherein the targeting moiety in the active configuration does not have the photolabile group.
35. The method of any of claims 33 or 34, wherein the photolabile group is a DMNPE group.
36. The method of claim 35, wherein the targeting moiety is a peptide and wherein the DMNPE is bound to the carboxylic acid terminus of the targeting moiety when the targeting moiety is in the inactive configuration.
37. The method of any of claims 22 to 36, wherein the light exposed to the nanocapsules has a wavelength between 300-450 nm.
38. A method of manufacturing a composition for delivering an agent to a cell, comprising:
forming a nanocapsule comprising one or more agents and a targeting molecule in an inactive configuration; wherein the targeting molecule switches from the inactive configuration to an active configuration in response to an external stimulus.
39. The method of claim 38, wherein the targeting moiety is a peptide comprising the amino acid sequence SEQ ID NO:1 (HAIYPRH).
40. The method of claim 38 or 39, wherein the targeting moiety in the inactive configuration comprises a photolabile group.
41. The method of any of claims 19 to 40, wherein the agent is one or more selected from the group consisting of a biologically active molecule, a drug, a cytokine or an RNA molecule.

1 of 3

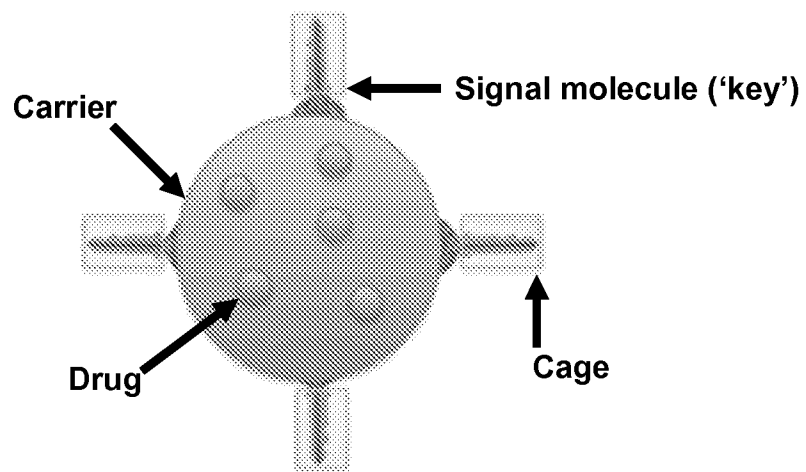


Figure 1

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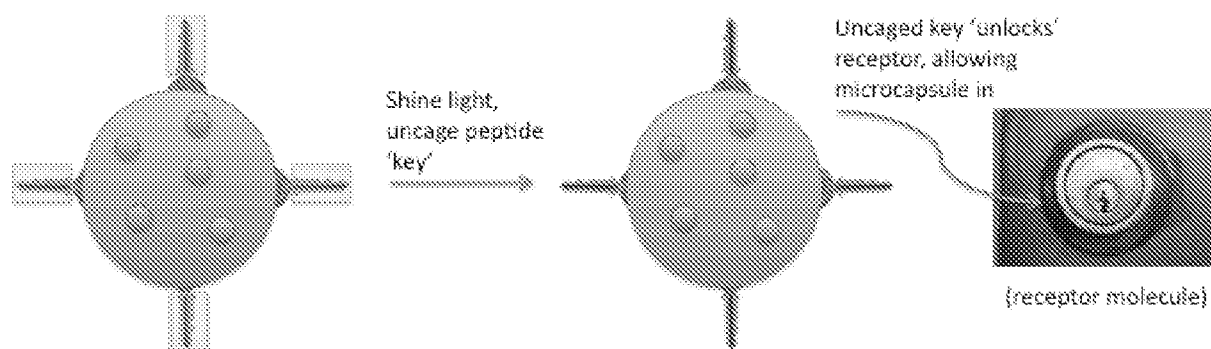


Figure 2

3 of 3

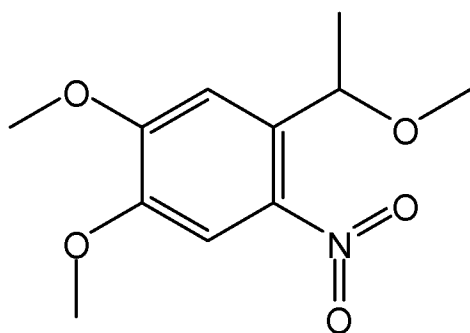


Figure 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2011/024087

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 47/00 (2006.01)

A61K 9/51 (2006.01)

A61K 47/42 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, Medline and EPODOC. Keywords: nanocapsule, nanoparticle, targeting moiety, haiyprh, transferrin, external stimulus, light, photolabile, dmnp, di-methoxy nitro phenyl ethyl, receptor, endocytosis, controlled delivery, targeted deliver and like terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DVIR, T et al. "Photo-Targeted Nanoparticles". NANO LETTERS, 2010. Vol.10(1): pages 250-254 See whole document, in particular Abstract, page 2, 1 st to 3 rd paragraphs; page 2, 6 th para.; page 3, 4 th para and Figure 1	1-3, 5, 6, 8, 11-13, 15-29, 31, 33, 34, 37 38, 40 & 41
Y	See as above; page 3, 3 rd para. and page 3, 4 th para.	4, 7, 9, 10, 14, 30, 32, 35, 36 & 39
X	TIMKO, B et al. "Remotely Triggerable Drug Delivery Systems". ADVANCED MATERIALS, 2010. Vol.22: pages 4925-4943 See whole document, in particular page 4932: 3.4. Phototargeted Nanoparticles and figure 5	1-3, 5, 6, 8, 11-13, 15, 16, 19, 38, 40 & 41

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

11 March 2011

Date of mailing of the international search report

16 MAR 2011

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2011/024087

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHAMAY, Y. et al. "Light Induced Drug Delivery Into Cancer Cells". BIOMATERIALS, February 2011 (available online November 2010). Vol.32: pages 1377-1386 See whole document, in particular abstract; page 1378, left col.; Fig.1 and page 1382, 2 nd last para.	1-3, 5, 6, 8, 11-29, 31, 33, 34, 37, 38, 40 & 41
Y	See as above and page 1384, left col.	4, 7, 9, 10, 30, 32, 35, 36 & 39
Y	US 2008/0166293 A1 (ENGLER et al) 10 July 2008 See whole document, in particular Abstract and claims	4, 7, 14, 30, 32 & 39
Y	US 6,017,758 A (HASELTON, III et al) 25 January 2000 See whole document, in particular col.4, lines 18-50 and claims	9, 10, 35 & 36
A	WO 2008/127743 A2 (WILLIAM MARSH RICE UNIVERSITY) 23 October 2008	
A	HAN, L et al. "Peptide-Conjugated PAMAM for Targeted Doxorubicin Delivery to Transferrin Receptor Overexpressed Tumors". MOLECULAR PHARMACEUTICS, 2010. Vol.7(6): pages 2156-2165 (Abstract Only)	
A	WO 2010/009106 A1 (BOURKE, Frederic A., Jr. and VO DINH, Tuan) 21 January 2010	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2011/024087

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
US 2008166293	AU 36499/02	AU 2002236499	CA 2449412
	US 2002115824	US 6743893	US 2006193778
	WO 0244329		
US 6017758	US 6242258	US 2001024830	US 6410327
WO 2008127743	US 2010040549		
WO 2010009106	AR 070461	US 2010003316	WO 2009105662
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			
END OF ANNEX			