blocked (embolized) to cut off the blood supply of the tumor. Prior to embolization, a chemotherapeutic agent is injected into the artery to deliver it preferentially to the tumor cells. This approach leaves the hepatic portal vein intact and is therefore thought to preserve the health of non-tumor liver cells that mainly depend on it for blood supply. Recently, the use of beads that release chemotherapeutic agents over time have been suggested to increase the effectiveness of these treatments.

[0006] Similarly, transarterial radioembolization uses the same types of particles to block the blood supply of the tumor; however, instead of chemotherapeutic agents, the particles rely on radiation given off by isotopes such as Yttrium-90 (Y-90) embedded in the particles (microspheres) that are delivered to the tumor. A variant on this procedure, known as percutaneous local ablation, follows the radioembolization with multiple days of direct injections of ethanol to the tumor.

[0007] Lastly, there is microwave ablation that uses electromagnetic waves with frequencies greater than 900 kHz to heat the tumor to a temperature higher than 100 °C. This allows for a faster and more uniform ablation of the tumor, but studies have yet to show any statistical difference in efficiency compared to radioembolization.

[0008] The standard of care for patients with HCC considered too advanced for resection or localized ablation is systemic chemotherapy. The only treatment that has shown an improvement in mean survival of treatment groups is Bayer's Nexavar (sorafenib), which only prolongs survival by three months. Thus, there is a need for additional treatment options for HCC and other cancers.

SUMMARY

[0008a] In one aspect, provided herein is a method for producing liposome containing alginate microspheres comprising: Atomizing, using an atomizer, a liposome/alginate solution into a curing solution comprising an alginate cross-linker, and isolating liposome containing alginate microsphere having an average diameter of 20 to 80 μm.

[0008b] In another aspect, provided herein is a liposome containing alginate microsphere comprising:

(a) an alginate microsphere having an average diameter of 20 to 80 μm; and

(b) liposomes dispersed in the alginate microsphere, the liposome containing a therapeutic and/or imaging agent.

[0008c] In another aspect, provided herein is the use of the liposome containing alginate microsphere described herein in the manufacture of a medicament for embolization therapy, wherein the medicament is to be injected into the tumor vasculature of a subject.

[0008d] In another aspect, provided herein is a thermotherapeutic alginate microsphere comprising a thermotherapeutic agent encapsulated in an alginate microsphere.

[0008e] In yet another aspect, provided herein is the liposome containing alginate microsphere described herein for use in therapy.

[0009] Certain examples are directed to compositions comprising and method for producing liposome containing alginate microspheres, optionally the liposomes encapsulate a variety of useful substances. Substances of note that can be encapsulated in liposomes and loaded into alginate microspheres include radiotherapeutics (*e.g.*, rhenium-188), radiolabels (*e.g.*, technetium-99m), chemotherapeutics (doxorubicin), magnetic particles (*e.g.*, 10 μm iron nanoparticles), and radio-opaque material (*e.g.*, iodine contrast). In certain examples, rhenium-188 liposomes in alginate microspheres (Rhe-LAMs) can be used for treatment of liver tumors, specifically hepatocellular carcinoma (HCC). In a more particular example HCC treatment can be through radioembolization, where the microspheres block the blood supply

to the tumor from the artery, while the rhenium-188 also delivers a high dose of radiation that is primarily targeted to the cancer cells.

[0010] Microparticles produced by standard production methods frequently have a wide particle size distribution, lack uniformity, fail to provide adequate release kinetics or other properties, and are difficult and expensive to produce. In addition, the microparticles may be large and tend to form aggregates, requiring a size selection process to remove particles considered to be too large for administration to patients by injection or inhalation. This requires sieving and results in product loss. Certain examples described herein use an ultrasonic nozzle or nebulizer to produce liposome containing microspheres. An ultrasonic nebulizer uses high-frequency electrical energy to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the liquid or formulation to form microspheres either directly or through a coupling fluid, creating an aerosol containing microspheres, which are subsequently cured or cross-linked. Typically, ultrasonic energy disrupts the association or lipids forming a liposome. The results described herein are surprising and unexpected in that the liposomes resist the disruptive effects of ultrasound remaining intact during production processes resulting in the formation of smaller liposome containing alginate microspheres.

[0011] In certain examples, liposome containing alginate microspheres (LAMs) are produce by spraying a liposome/alginate solution (liquid or feed source) into a curing solution having an alginate cross-linker. Typically, a liquid is supplied by powered pumps to simple or complex orifice nozzles that atomize the liquid stream into spray droplets that are cross-linked when exposed to the curing solution. Nozzles are often selected primarily on the desired range of flow rates needed and secondarily on the range of liquid droplet size. Any spray atomizer that can produce droplets from the liquids described herein can be used. Suitable spray atomizers include two-fluid nozzles, single fluid nozzles, ultrasonic nozzles such as the Sono-Tek™ ultrasonic nozzle, rotary atomizers or vibrating orifice aerosol generators (VOAG), and the like. In certain examples, the nozzle is an ultrasonic nozzle, a 1 Hz to about 100 kHz nozzle. In one particular example the nozzle is a 25 kHz nozzle. In certain examples, the spray atomizer can have one or more of the following specifications. (a) a 25kHz to 180kHz nozzle, in particular a 25 kHz nozzle. (b) a 1 to 10 W generator, in particular a 5.0 W generator. (c) a pump capable of a flow rate of 0.1 to 1.0 ml/min, in particular 0.5 ml/min (microbore may be necessary for a flow rate this low). The curing

solution can be positioned to receive the atomized liquid. The distance between the nozzle and the curing solution can be varied between 1 to 10 cm, in particular 4 cm. the system can be activated for the entirety of nozzle usage. The generator can be activated and the pump can form liposome containing alginate microspheres (LAMs). Microspheres can be incubated at room temp (*e.g.*, 20 to 30 °C) in the curing solution (*e.g.*, $CaCl_2$ solution) for 1 to 10 minutes, in particular 5 minutes. In certain examples, the microspheres can be spun down, for example at 1000-1200 rpm. Followed by abstracting the supernatant to wash the spheres of free reagents, *e.g.*, unbound Re-188/Tc-99m. Microsphere solution can be passed through a 100 µm-pore stainless steel mesh for exclusion of any clumping that may have occurred during the cross-linking or centrifugation. These LAMs can be used for intraarterial administration. In certain examples, the microspheres can be visualized under light microsopy, and dosimeter can be used to measure radioactivity retention in those LAMs loaded with radioactive materials.

[0012] Certain examples are directed to LAMs having a diameter of 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 350, 400, 450, to 500 µm, including all values and ranges there between (in certain examples any of the values or subranges can be specifically excluded). In certain examples, the LAMs have an average diameter of 20 to 80 µm, including all values and ranges there between. In certain examples the ratio of liposome to alginate (w/w or v/v) is 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, including all ratios and ranges there between (in certain examples any of the values or subranges can be specifically excluded). In certain examples, the LAM comprises 10 to 80 weight percent liposome/lipid, 10 to 80 weight percent alginate solution, 0.01 to 5 weight percent alginate cross-linker, and 1 to 30 weight percent therapeutic and/or imaging agent.

[0013] As used herein, a “liposome” refers to a vesicle consisting of an aqueous core enclosed by one or more phospholipid layers. Liposomes may be unilamellar, composed of a single bilayer, or they may be multilamellar, composed of two or more concentric bilayers. Liposomes range from small unilamellar vesicles (SUVs) to larger multilamellar vesicles. LMVs form spontaneously upon hydration with agitation of dry lipid films/cakes which are generally formed by dissolving a lipid in an organic solvent, coating a vessel wall with the solution and evaporating the solvent. Energy is then applied to convert the LMVs to SUVs, LUVs, etc. The energy can be in the form of, without limitation, sonication, high pressure, elevated temperatures and extrusion to provide smaller single and multi-lamellar vesicles.

During this process some of the aqueous medium is entrapped in the vesicle. Liposomes can also be prepared using emulsion templating. Emulsion templating comprises, in brief, the preparation of a water-in-oil emulsion stabilized by a lipid, layering of the emulsion onto an aqueous phase, centrifugation of the water/oil droplets into the water phase and removal of the oil phase to give a dispersion of unilamellar liposomes. Liposomes prepared by any method, not merely those described above, may be used in the compositions and methods of this disclosure. Any of the preceding techniques as well as any others known in the art or as may become known in the future may be used as compositions of therapeutic agents in or on a delivery interface of this disclosure. Liposomes comprising phospholipids and/or sphingolipids may be used to deliver hydrophilic (water-soluble) or precipitated therapeutic compounds encapsulated within the inner liposomal volume and/or to deliver hydrophobic therapeutic agents dispersed within the hydrophobic bilayer membrane. In certain examples the liposome comprises lipids selected from sphingolipids, ether lipids, sterols, phospholipids, phosphoglycerides, and glycolipids. In certain examples, the lipid includes, for example, DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine).

[0014] As used herein, “alginate” refers to a linear polysaccharide that can be derived from seaweed. The most common source of alginate is the species *Macrocystis pyrifera*. Alginate is composed of repeating units of D-mannuronic (M) and L-guluronic acid (G), presented in both alternating blocks and alternating individual residues. Soluble alginate may be in the form of monovalent salts including, without limitation, sodium alginate, potassium alginate and ammonium alginate. In certain examples, the alginate includes, but is not limited to one or more of sodium alginate, potassium alginate, calcium alginate, magnesium alginate, ammonium alginate, and triethanolamine alginate. Alginates are present in the formula in amounts ranging from 5 to 80% by weight, preferably in amounts ranging from 20 to 60% by weight, and most preferably about 50% by weight. In certain examples, the alginate is ultra-pure alginate (*e.g.*, Novamatrix ultra-pure alginate). Alginate can be cross-linked using ionic gelation provided through multivalent cations in solution, *e.g.*, an aqueous or alcoholic solution with multivalent cations therein, reacting with alginates. Multivalent cations (*e.g.*, divalent cations, monovalent cations are not sufficient for cross-linking alginate) for use with alginates include, but are not limited to calcium, strontium, barium, iron, silver, aluminum, magnesium, manganese, copper, and zinc, including salts thereof. In certain examples, the cation is calcium and is provided in the form of an aqueous calcium chloride solution.

[0015] In certain examples the therapeutic or imaging agent is a chemotherapeutic, radiotherapeutic, thermotherapeutic, or a contrast agent.

[0016] In certain examples, a radiotherapeutic agent includes a radiolabel such as a beta emitter (^{131}I, ^{90}Y, ^{177}Lu, ^{186}Re, ^{188}Re, any one of which can be specifically excluded) or gamma emitter (^{125}I, ^{123}I). In certain examples, the radiotherapeutic agent is ^{188}Re. Furthermore, the term "radiotherapeutic" may be taken to more broadly encompass any radioactively-labeled moiety, and may include any liposome or LAM associated with or comprising a radionuclide. The liposome or LAM may be associated with a radionuclide through a chelator, direct chemical bonding, or some other means such as a linker protein.

[0017] In certain examples, a chemotherapeutic agent includes, but is not limited to a chemical compound that inhibits or kills growing cells and which can be used or is approved for use in the treatment of cancer. Exemplary chemotherapeutic agents include cytostatic agents which prevent, disturb, disrupt or delay cell division at the level of nuclear division or cell plasma division. Such agents may stabilize microtubules, such as taxanes, in particular docetaxel or paclitaxel, and epothilones, in particular epothilone A, B, C, D, E, and F, or may destabilize microtubules such as vinca alcaloids, in particular vinblastine, vincristine, vindesine, vinflunine, and vinorelbine. Liposome can be used to carry hydrophilic agents as micelles can be used to carry lipophilic agents.

[0018] In general, the thermotherapeutic agents include a plurality of magnetic nanoparticles, or "susceptors," of an energy susceptive material that are capable of generating heat via magnetic hysteresis losses in the presence of an energy source, such as, an alternating magnetic field (AMF). The methods described herein, generally, include the steps of administering an effective amount of a thermotherapeutic compound to a subject in need of therapy and applying energy to the subject. The application of energy may cause inductive heating of the magnetic nanoparticles which in turn heats the tissue to which the thermotherapeutic compounds were administered sufficiently to ablate tissue. In certain examples, a thermotherapeutic agent includes, but is not limited to magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) and $FeCo/SiO_2$, and in some examples, may include aggregates of superparamagnetic grains of, for example, $Co_{36}C_{65}$, $Bi_3Fe_5O_{12}$, $BaFe_{12}O_{19}$, NiFe, CoNiFe, Co-Fe_3O_4, and FePt-Ag, where the state of the aggregate may induce magnetic blocking. In thermotherapy, the response of MNPs to AC magnetic field causes thermal energy to be dissipated into the surroundings, killing the tumor cells. Additionally, hyperthermia can

enhance radiation and chemotherapy treatment of cancer. The term “hyperthermia”, as used herein, refers to heating of tissue to temperatures between about 40° C. and about 60° C. The term “alternating magnetic field” or “AMF”, as used herein, refers to a magnetic field that changes the direction of its field vector periodically, typically in a sinusoidal, triangular, rectangular or similar shape pattern, with a frequency of in the range of from about 80 kHz to about 800 kHz. The AMF may also be added to a static magnetic field, such that only the AMF component of the resulting magnetic field vector changes direction. It will be appreciated that an alternating magnetic field may be accompanied by an alternating electric field and may be electromagnetic in nature. In certain examples, the thermotherapeutic agent can be incorporated into alginate microspheres in the absence of lipids and as such form a thermotherpeutic containing alginate microsphere where the agent is not incorporated in a liposome but is incorporated in the alginate microsphere.

[0019] In certain examples, a contrast or imaging agent includes, but is not limited a transition metal, carbon nanomaterials such as carbon nanotubes, fullerene and graphene, near-infrared (NIR) dyes such as indocyanine green (ICG), and gold nanoparticles. Transition metal refers to a metal in Group 3 to 12 of the Periodic Table of Elements, such as titanium (Ti), vanadium (V), niobium (Nb), tantalum (Ta), chromium (Cr), molybdenum (Mo), tungsten (W), manganese (Mn), iron (Fe), ruthenium (Ru), osmium (Os), iridium (Ir), nickel (Ni), copper (Cu), technetium (Tc), rhenium (Re), cobalt (Co), rhodium (Rh), iridium (Ir), palladium (Pd), platinum (Pt), silver (Ag), gold (Au), a lanthanide such as europium (Eu), gadolinium (Gd), lanthanum (La), ytterbium (Yb), and erbium (Er), or a post-transition metal such as gallium (Ga), and indium (In). In one example, the imaging modality is selected from the group comprising, Positron Emission Tomography (PET), Single Photon Emission Tomography (SPECT), Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Ultrasound Imaging (US), and Optical Imaging. In another example of the disclosure, the imaging modality is Positron Emission Tomography (PET). The imaging agent includes, but is not limited to a radiolabel, a fluorophore, a fluorochrome, an optical reporter, a magnetic reporter, an X-ray reporter, an ultrasound imaging reporter or a nanoparticle reporter. In another example of the disclosure, the imaging agent is a radiolabel selected from the group comprising a radioisotopic element selected from the group consisting: of astatine, bismuth, carbon, copper, fluorine, gallium, indium, iodine, lutetium, nitrogen, oxygen, phosphorous, rhenium, rubidium, samarium, technetium, thallium, yttrium, and zirconium. In another example, the radiolabel is selected from the group comprising zirconium-89 (^{89}Zr), iodine-

124 (^{124}I), iodine-131 (^{131}I), iodine-125 (^{125}I) iodine-123 (^{123}I), bismuth-212 (^{212}Bi), bismuth-213 (^{213}Bi), astatine-221 (^{211}At), copper-67 (^{67}Cu), copper-64 (^{64}Cu), rhenium-186 (^{186}Re), rhenium-186 (^{188}Re), phosphorus-32 (^{32}P), samarium-153 (^{153}Sm), lutetium-177 (^{117}Lu), technetium-99m (^{99m}Tc), gallium-67 (^{67}Ga), indium-111 (^{111}In), thallium-201 (^{201}Tl) carbon-11, nitrogen-13 (^{13}N), oxygen-15 (^{15}O), fluorine-18 (^{18}F), and rubidium-82 (^{82}Ru).

[0020] Other examples of the disclosure are discussed throughout this application. Any example discussed with respect to one example of the disclosure applies to other examples of the disclosure as well and vice versa. Each example described herein is understood to be examples of the disclosure that are applicable to all examples of the disclosure. It is contemplated that any example discussed herein can be implemented with respect to any method or composition of the disclosure, and vice versa. Furthermore, compositions and kits of the disclosure can be used to achieve methods of the disclosure.

[0021] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[0022] Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0023] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

[0024] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0025] As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having," "contains", "containing," "characterized by" or any other variation thereof, are intended to encompass a non-exclusive inclusion, subject to any limitation explicitly indicated otherwise, of the recited components. For example, a chemical composition and/or

method that “comprises” a list of elements (e.g., components or features or steps) is not necessarily limited to only those elements (or components or features or steps), but may include other elements (or components or features or steps) not expressly listed or inherent to the chemical composition and/or method.

[0026] As used herein, the transitional phrases “consists of” and “consisting of” exclude any element, step, or component not specified. For example, “consists of” or “consisting of” used in a claim would limit the claim to the components, materials or steps specifically recited in the claim except for impurities ordinarily associated therewith (i.e., impurities within a given component). When the phrase “consists of” or “consisting of” appears in a clause of the body of a claim, rather than immediately following the preamble, the phrase “consists of” or “consisting of” limits only the elements (or components or steps) set forth in that clause; other elements (or components) are not excluded from the claim as a whole.

[0027] As used herein, the transitional phrases “consists essentially of” and “consisting essentially of” are used to define a chemical composition and/or method that includes materials, steps, features, components, or elements, in addition to those literally disclosed, provided that these additional materials, steps, features, components, or elements do not materially affect the basic and novel characteristic(s) of the claimed disclosure. The term “consisting essentially of” occupies a middle ground between “comprising” and “consisting of”.

[0028] Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific examples of the disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of the specification embodiments presented herein.

CLAIMS

1. A method for producing liposome containing alginate microspheres comprising: Atomizing, using an atomizer, a liposome/alginate solution into a curing solution comprising an alginate cross-linker, and isolating liposome containing alginate microsphere having an average diameter of 20 to 80 μm.

2. The method of claim 1, wherein the atomizer is an ultrasonic nozzle.

3. The method of claim 2, wherein the ultrasonic nozzle is a 1 Hz to 100 kHz nozzle.

4. The method of claim 2, wherein the ultrasonic nozzle is a 25 kHz nozzle.

5. The method of claim 1, wherein the atomizer is positioned 1 to 10 cm from the curing solution.

6. The method of claim 1, wherein the curing solution comprises a cation.

7. The method of claim 6, wherein the cation is selected from calcium, strontium, barium, iron, silver, aluminum, magnesium, manganese, copper, and zinc.

8. The method of claim 1, wherein the curing solution comprises $CaCl_2$.

9. The method of claim 1, wherein the liposome/alginate solution comprises a liposome to alginate ratio of 1:1.

10. The method of claim 1, wherein the liposomes comprise a therapeutic agent or an imaging agent.

11. The method of claim 10, wherein the therapeutic agent is a thermotherapeutic, a chemotherapeutic, or a radiotherapeutic agent.

12. A liposome containing alginate microsphere comprising:

(a) an alginate microsphere having an average diameter of 20 to 80 μm; and

(b) liposomes dispersed in the alginate microsphere, the liposome containing a therapeutic and/or imaging agent.

13. Use of the liposome containing alginate microsphere of claim 12 in the manufacture of a medicament for embolization therapy, wherein the medicament is to be injected into the tumor vasculature of a subject.

14. A thermotherapeutic alginate microsphere comprising a thermotherapeutic agent encapsulated in an alginate microsphere.

15. The microsphere of claim 14, wherein the thermotherpeutic agent is encapsulated in a liposome that is contained in an alginate microsphere.

16. The liposome containing alginate microsphere of claim 12 for use in therapy.