(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 May 2009 (07.05.2009)

PCT

(10) International Publication Number
WO 2009/058134 A1

(51) International Patent Classification:
C09D 185/02 (2006.01)

(21) International Application Number:
PCT/US2007/083043

(22) International Filing Date: 30 October 2007 (30.10.2007)

(25) Filing Language: English

(26) Publication Language: English

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(54) **Title:** LOADABLE POLYMERIC PARTICLES FOR MARKING OR MASKING INDIVIDUALS AND METHODS OF PREPARING AND USING THE SAME

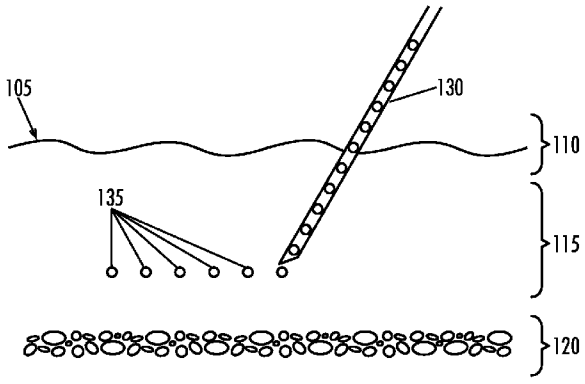

Fig. 12

(57) **Abstract:** The present invention relates to the use of certain microspheres, nanospheres, and other structures to provide a method of marking or masking identifying marks in individual biological hosts. Biological hosts for the present invention may include humans, other animals, or plants. Such methods may be used to sense, signal, track, mark, or identify individual biological hosts. Microspheres, nanospheres, and other structures of the present invention may be implanted, injected, ingested, or attached to individual biological hosts. Microspheres, nanospheres, and other structures of the present invention comprise poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof which may be present throughout the particles or within an outer coating of the particles. The microspheres, nanospheres, and other structures may also comprise a core having a hydrogel which may further comprise one or more dyes or other chromophoric agents covalently bound permanently to the hydrogel core material. The microspheres, nanospheres, and other structures and/or the hydrogel core may further comprise radio frequency or other electronic chips or nanochips, capable of transmitting and/or receiving electronic signals from external transmitters and/or receivers.

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LOADABLE POLYMERIC PARTICLES FOR MARKING OR MASKING INDIVIDUALS AND METHODS OF PREPARING AND USING THE SAME

BACKGROUND OF THE INVENTION

[0001] In selected clinical applications, it may be advantageous to provide microspheres, nanospheres, and other structures to provide a method of marking or masking identifying marks in individual biological hosts. Biological hosts for the present invention may include humans, other animals, or plants. Such methods may be used to sense, signal, track, mark, or identify individual animals or plants. Microspheres, nanospheres, and other structures of the present invention may be implanted, injected, ingested, or attached to individual biological hosts.

[0002] Many situations exist in which the ability to identify with certainty and speed a particular individual biological host, or to locate a certain site on the body of a particular individual biological host is of value. Examples of such situations include, but are not limited to, the needs to identify an individual with a particular medical implant, to identify a particular individual as part of a herd vaccination program, or to identify an individual for security purposes. Additionally, a need exists to be able to mask existing anatomic marks, such as cosmetic treatment to hide a scar or prior tattoo.

[0003] Existing identification techniques for mammals include the use of physically applied tags, brands, tattoos, or implantable electronic devices. Such techniques have their individual disadvantages: external tags may be dislodged and lost; branding is inhumane; both branding and tattooing creates visible marks that mar the skin surface; and implantable devices require surgical placement. Existing techniques are thus less than optimal for use in marking livestock or other animals for identification, and are wholly unsuited for use in marking humans.

[0004] In addition, a need exists for improved means of masking or removing such markings as tattoos when desired. Existing techniques such as surgical excision,

dermabrasion, or laser ablation have cosmetic and functional limitations, and do not always provide reliable and economical ways of removing such markings when desired, either for functional purposes or cosmetic considerations.

[0005] Most prior art particles used in medical applications are characterized by numerous disadvantages including irritation of the tissues with which they come in contact and initiation of adverse immune reactions. Additionally, many of the materials used to prepare the prior art particles may degrade relatively rapidly within the mammalian body, thereby detracting from their utility in certain procedures where long term presence of intact particles may be necessary. Moreover, the degradation of the prior art materials may release toxic or irritating compounds causing adverse reactions in the patients.

[0006] It is also a problem in the art for certain types of prior art particles that it is difficult to achieve desirable suspension properties when the particles are incorporated into a delivery suspension for injection into a site in the body to be treated. Many times, the particles settle out or tend to "float" in the solution such that they are not uniformly suspended for even delivery. Furthermore, particles may tend to aggregate within the delivery solution and/or adhere to some part of the delivery device, making it necessary to compensate for these adhesive/attractive forces.

[0007] In order to achieve a stable dispersion, it is known to add suitable dispersing agents that may include surfactants directed at breaking down attractive particle interaction. Depending on the nature of the particle interaction, the following materials may be used: cationic, anionic or nonionic surfactants such as Tween™ 20, Tween™ 40, Tween™ 80, polyethylene glycols, sodium dodecyl sulfate, various naturally occurring proteins such as serum albumin, or any other macromolecular surfactants in the delivery formulation. Furthermore thickening agents can be used help prevent particles from settling by sedimentation and to increase solution viscosity, for example, polyvinyl alcohols, polyvinyl pyrrolidones, sugars or dextrins. Density additives may also be used to achieve buoyancy.

[0008] It can also be difficult to visualize microparticles in solution to determine their degree of suspension when using clear, transparent polymeric acrylate hydrogel beads in aqueous suspension. Attempts to use the inert precipitate, barium sulfate, in particle form is known as an additive for bone cement, for silicones for rendering items visible during X-

ray examination and for providing radiopacity to polymeric acrylate particles. See Jayakrishnan et al., Bull. Mat. Sci., Vol. 12, No. 1, pp. 17-25 (1989). The barium sulfate also is known for improving fluidization, and is often used as an inorganic filler to impart anti-stick behavior to moist, aggregated particles. Other prior art attempts to increase visualization of microparticles include use of gold, for example, Embosphere Gold™ provides a magenta color to acrylate microparticles using small amounts of gold.

[0009] In certain medical applications, it may further be of value to provide microparticles such as microspheres in one or more sizes. Furthermore, it may also be of value to a user to provide each of such sizes of microspheres incorporated with color-coded associated dyes to indicate the microsphere size to the user. In various applications, the size of the microspheres could correspond to differing doses of active marking or masking agents contained within the microspheres. In yet other applications of use, it may further be of value to provide sized and color-coded microspheres to a user in similarly color-coded syringes or other containers for transport and delivery to further aid a user in identifying the size of microspheres being used. In yet other applications, the inclusions of marking or masking agents such as dyes visible only under certain inducing conditions would be of value to provide a means of visualizing such particles only when desired.

[0010] There thus exists in the art a need for small particles that can be formed to have a preferential generally spherical configuration for certain applications such as various therapeutic and diagnostic procedures which are not degraded by the natural systems of the mammalian system, are biocompatible, are easy to visualize in suspension while in use and/or demonstrate acceptable physical and suspension properties.

[0011] At the same time, in other medical applications, the need also exists for small particles that can be formed to have a preferential generally spherical configuration for certain temporary identification applications which are degraded by the natural systems of the mammalian system, are biocompatible, may be easily visualized in suspension while in use and/or demonstrate acceptable physical and suspension properties.

BRIEF SUMMARY OF THE INVENTION

[0012] The invention includes a particle for use as a means of providing identification and/or a desired marking or masking of identifying marks of individual biological hosts. The particle comprises poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof.

[0013] The invention further includes a particle for use as a means of visually masking an existing marking in individual biological hosts. The particle comprises poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof.

[0014] The present invention further includes particles comprising poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof provided as microspheres or other shapes provided in one or more specified sizes.

[0015] The present invention further includes particles comprising poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof provided as sized microspheres or other shapes and further comprising a color-coded dye incorporated into or attached to the exterior of the microspheres to visually aid a user in identifying the size of microspheres in use.

[0016] The present invention further includes particles comprising poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof provided as sized microspheres or other shapes and further comprising materials or devices imbedded or encapsulated with poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof, such materials or devices being capable of responding to an externally applied stimulus.

[0017] Microspheres of the present invention may further be provided as sized microspheres further comprising a color-coded dye incorporated into or attached to the exterior of the microspheres and contained or delivered in a similarly color-coded syringe or other transport or delivery container to further visually aid a user in providing a visual confirmation of the specific size of microspheres in use.

[0018] Microspheres of the present invention may further be provided as sized microspheres further comprising a colored dye incorporated into or attached to the exterior of the microspheres and contained or delivered in a similarly color-coded syringe or other

transport or delivery container to functionally serve to impart a desired color to mammalian tissues in use.

[0019] Further described herein is a method of delivering an active marking agent to a localized area within a body of a mammal comprising contacting the localized area with at least one of a particle comprising poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof and an active marking agent, such that an effective amount of the active agent is delivered to the localized area.

[0020] Yet further described herein is a method of delivering an active masking agent to a localized area within a body of a mammal comprising contacting the localized area with at least one of a particle comprising poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof and an active masking agent, such that an effective amount of the active agent is delivered to the localized area.

[0021] Also within the invention is a sustained release formulation of an active marking or masking agent, the formulation comprising a polymer capsule and an active marking or masking agent, wherein the polymeric capsule comprises poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof.

[0022] The invention also includes a method of delivering an active marking or masking agent to a localized area within the body of a mammal comprising contacting the localized area with at least one of a particle comprising poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof and an active marking or masking agent, such that an effective amount of the active marking or masking agent is exposed to the localized area, wherein the particle may further comprise an agent to increase density.

[0023] Further, a method for minimizing agglomeration of particles formed from acrylic-based polymers is described in which the method comprises providing barium sulfate to the core and/or surface of the particles.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0024] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings.

For the purpose of illustrating the invention, there are shown in the drawings embodiments that are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown.

[0025] In the drawings:

[0026] Fig. 1 shows a schematic representation of a general cryoextraction scheme used to prepare particles according to one embodiment of the invention;

[0027] Fig. 2 shows the manual dripping technique by which the polymer solution was supplied to liquid nitrogen in preparation of the microspheres of Example 1, herein;

[0028] Fig. 3A and Fig. 3B show unloaded polyphosphazene particles (microspheres) as prepared by one embodiment of the cryoextraction method as described herein. Figure 3A shows a 4x optical microscope view and Fig. 3B shows a 100x scanning electron microscope view;

[0029] Fig. 4 shows a particle (microsphere) formed according to one embodiment of the invention loaded with bovine insulin (20% (wt/wt)) at 100x magnification SEM;

[0030] Fig. 5A and Fig. 5B show the surface morphology of unloaded polyphosphazene microspheres. Fig. 5A is an image obtained using an atomic force microscope and Fig. 5B is a scanning electron micrograph showing the surface of an unloaded polyphosphazene microsphere at 5000x magnification;

[0031] Figs. 6 and 7 show a cryoextraction setup for use in an embodiment of the invention wherein Fig. 6 is a cryoextraction vessel and Fig. 7 is a syringe pump;

[0032] Fig. 8 is a cross-sectional view of an apparatus for use in microcatheter testing of microparticles in Example 14 herein;

[0033] Figs 9A and 9B show an SEM at 1.0KX magnification of the surface of the Sample C microparticles just after the hydration/dehydration cycle and at a 50.00KX magnification of the film thickness of microparticles formed in accordance with Sample C of Example 12 used in the evaluation of Example 14, respectively;

[0034] Figs. 10A, 10B, 10C and 10D are SEMs of microparticles made in accordance with Sample C of Example 12 used in the evaluation of Example 14 after passing through a catheter showing surface features (Figs. 10A, 10B and 10C) at 1.0KX magnification and at 5.0KX magnification (Fig. 10D); and

[0035] Figs. 11A, 11B, 11C and 11D are SEMs of microparticles formed in accordance with Sample C of Example 12 after thermal stress testing in Example 14. Fig. 11A is a 50X magnification of a minor amount of delamination in the strong white contrast portion. Fig. 11B is a 200X magnification of the microparticles of Fig. 11A. Figs. 11C and 11D are, respectively, 200X and 1.0KX magnified SEMs of other Sample C microparticles showing only minor defects.

[0036] Fig. 12 shows a cross section of skin, in which a pattern of microspheres according to the present invention are being delivered by an intradermal needle injection.

[0037] Fig. 13 shows a cross section of skin containing an intradermal microparticle according to the present invention comprising a polymer shell and an encased radio frequency identification chip.

[0038] Fig. 14 shows a cross section of skin containing a previous tattoo with intradermal pigmentation, in which a pattern of microspheres according to the present invention are being delivered by an intradermal needle injection superficial to the pigmentation to mask the prior tattoo.

[0039] Fig. 15 shows a schematic view of an automatic inoculation system which senses the identifying microparticles of the present invention and integrates with a computer controlled delivery system to provide one or more inoculations specific to an identified individual's needs.

DETAILED DESCRIPTION OF THE INVENTION

[0040] Described herein are particles that may be manufactured using poly[bis(trifluoroethoxy) phosphazene] and/or derivatives thereof, as well as methods of preparing such particles. Additionally, described herein are therapeutic methods and procedures which use the particles as described herein, including methods of delivery of an

active marking or masking agent using the particle locally to mark or identify individual biological hosts.

[0041] Also included are sustained release drug delivery formulations for implantable or injectable administration including the particles for localized delivery of an active marking or masking agent to the integument and/or systemic delivery of an active agent as well as a sustained release delivery formulation that can be injected intradermally to impart a desired marking to a desired individual biological host for a desired period of time during the release of the active marking or masking agent .

[0042] All of the methods, compositions and formulations of the invention utilize at least one particle as described herein. "Particle" and "particles" as used herein mean a substantially spherical or ellipsoid article(s), hollow or solid, that may have any diameter suitable for use in the specific methods and applications described below, including a microsphere(s) and a nanosphere(s), beads and other bodies of a similar nature known in the art.

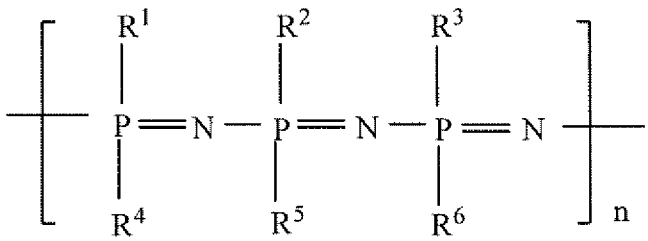
[0043] The preferred particles of the invention according to one embodiment described herein are composed, in whole or in part, the specific polyphosphazene polymer known as poly[bis(trifluoroethoxy)phosphazene] or a derivative of poly[bis(trifluoroethoxy)phosphazene]. Use of this specific polymer provides particles that are at least in part inorganic in that they include an inorganic polymer backbone and which are also biocompatible in that when introduced into a mammal (including humans and animals), they do not significantly induce a response of the specific or non-specific immune systems.

[0044] The particles are useful in a variety of identification or masking procedures in part because they can be prepared in a variety of sizes and colors for various purposes. Additionally, owing to the biocompatible nature of the polymer, the particles facilitate avoidance or elimination of immunogenic reactions generally encountered when foreign bodies are introduced into a biological host body, such as "implant rejection" or "allergic shock," and other adverse reactions of the immune system. Moreover, it has been found that the particles of the invention may be provided in a form to exhibit reduced biodegradation *in vivo,* thereby increasing the long-term stability of the particle in the biological environment. Moreover, in those situations where some degradation is

undergone by the polymer in the particle, the products released from the degradation include only non-toxic concentrations of phosphorous, ammonia, and trifluoroethanol, which, advantageously, is known to promote anti-inflammatory responses when in contact with biological host tissue.

[0045] Reduction and/or elimination of immunogenic reactions is particularly important in restorative applications, where scarring and tissue edema are particularly undesirable, where secondary tissue reactions may distort or defeat the purpose of a marking or masking implant or injection..

[0046] Each of the particles in the invention is formed at least in part of the polymer, poly[bis(2,2,2-trifluoroethoxy)phosphazene] or a derivative thereof (referred to further herein as "poly[bis(trifluoroethoxy)phosphazene]". As described herein, the polymer poly[bis(2,2,2-trifluoroethoxy)phosphazene] or derivatives thereof have chemical and biological qualities that distinguish this polymer from other know polymers in general, and from other know polyphosphazenes in particular. In one aspect of this invention, the polyphosphazene is poly[bis(2,2,2-trifluoroethoxy)phosphazene] or derivatives thereof, such as other alkoxide, halogenated alkoxide, or fluorinated alkoxide substituted analogs thereof. The preferred poly[bis(trifluoroethoxy)phosphazene] polymer is made up of repeating monomers represented by the formula (I) shown below:



(I),

wherein R^1 to R^6 are all trifluoroethoxy (OCH_2CF_3) groups, and wherein n may vary from at least about 40 to about 100,000, as disclosed herein. Alternatively, one may use derivatives of this polymer in the present invention. The term "derivatives" is meant to refer to polymers made up of monomers having the structure of formula I but where one or more of the R^1 to R^6 functional group(s) is replaced by a different functional group(s), such as an unsubstituted alkoxide, a halogenated alkoxide, a fluorinated alkoxide, or any combination thereof, or where one or more of the R^1 to R^6

is replaced by any of the other functional group(s) disclosed herein, but where the biological inertness of the polymer is not substantially altered.

[0047] In one aspect of the polyphosphazene of formula (I) illustrated above, for example, at least one of the substituents R^1 to R^6 can be an unsubstituted alkoxy substituent, such as methoxy (OCH_3), ethoxy (OCH_2CH_3) or n-propoxy ($OCH_2CH_2CH_3$). In another aspect, for example, at least one of the substituents R^1 to R^6 is an alkoxy group substituted with at least one fluorine atom. Examples of useful fluorine-substituted alkoxy groups R^1 to R^6 include, but are not limited to OCF_3, OCH_2CF_3, $OCH_2CH_2CF_3$, $OCH_2CF_2CF_3$, $OCH(CF_3)_2$, $OCCH_3(CF_3)_2$, $OCH_2CF_2CF_2CF_3$, $OCH_2(CF_2)_3CF_3$, $OCH_2(CF_2)_4CF_3$, $OCH_2(CF_2)_5CF_3$, $OCH_2(CF_2)_6CF_3$, $OCH_2(CF_2)_7CF_3$, $OCH_2CF_2CHF_2$, $OCH_2CF_2CF_2CHF_2$, $OCH_2(CF_2)_3CHF_2$, $OCH_2(CF_2)_4CHF_2$, $OCH_2(CF_2)_5CHF_2$, $OCH_2(CF_2)_6CHF_2$, $OCH_2(CF_2)_7CHF_2$, and the like. Thus, while trifluoroethoxy (OCH_2CF_3) groups are preferred, these further exemplary functional groups also may be used alone, in combination with trifluoroethoxy, or in combination with each other. In one aspect, examples of especially useful fluorinated alkoxide functional groups that may be used include, but are not limited to, 2,2,3,3,3-pentafluoropropyloxy ($OCH_2CF_2CF_3$), 2,2,2,2',2',2'-hexafluoroisopropyloxy ($OCH(CF_3)_2$), 2,2,3,3,4,4,4-heptafluorobutyloxy ($OCH_2CF_2CF_2CF_3$), 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyloxy ($OCH_2(CF_2)_7CF_3$), 2,2,3,3,-tetrafluoropropyloxy ($OCH_2CF_2CHF_2$), 2,2,3,3,4,4-hexafluorobutyloxy ($OCH_2CF_2CF_2CHF_2$), 3,3,4,4,5,5,6,6,7,7,8,8-dodecafluorooctyloxy ($OCH_2(CF_2)_7CHF_2$), and the like, including combinations thereof.

[0048] Further, in some embodiments, 1% or less of the R^1 to R^6 groups may be alkenoxy groups, a feature that may assist in crosslinking to provide a more elastomeric phosphazene polymer. In this aspect, alkenoxy groups include, but are not limited to, $OCH_2CH{=}CH_2$, $OCH_2CH_2CH{=}CH_2$, allylphenoxy groups, and the like, including combinations thereof. Also in formula (I) illustrated herein, the residues R^1 to R^6 are each independently variable and therefore can be the same or different.

[0049] By indicating that n can be as large as ∞ in formula I, it is intended to specify values of n that encompass polyphosphazene polymers that can have an average molecular weight of up to about 75 million Daltons. For example, in one aspect, n can

vary from at least about 40 to about 100,000. In another aspect, by indicating that n can be as large as ∞ in formula I, it is intended to specify values of n from about 4,000 to about 50,000, more preferably, n is about 7,000 to about 40,000 and most preferably n is about 13,000 to about 30,000.

[0050] In another aspect of this invention, the polymer used to prepare the polymers disclosed herein has a molecular weight based on the above formula, which can be a molecular weight of at least about 70,000 g/mol, more preferably at least about 1,000,000 g/mol, and still more preferably a molecular weight of at least about $3x10^6$ g/mol to about $20x10^6$ g/mol. Most preferred are polymers having molecular weights of at least about 10,000,000 g/mol.

[0051] In a further aspect of the polyphosphazene formula (I) illustrated herein, n is 2 to ∞, and R^1 to R^6 are groups which are each selected independently from alkyl, aminoalkyl, haloalkyl, thioalkyl, thioaryl, alkoxy, haloalkoxy, aryloxy, haloaryloxy, alkylthiolate, arylthiolate, alkylsulphonyl, alkylamino, dialkylamino, heterocycloalkyl comprising one or more heteroatoms selected from nitrogen, oxygen, sulfur, phosphorus, or a combination thereof, or heteroaryl comprising one or more heteroatoms selected from nitrogen, oxygen, sulfur, phosphorus, or a combination thereof. In this aspect of formula (I), the pendant side groups or moieties (also termed "residues") R^1 to R^6 are each independently variable and therefore can be the same or different. Further, R^1 to R^6 can be substituted or unsubstituted. The alkyl groups or moieties within the alkoxy, alkylsulphonyl, dialkylamino, and other alkyl-containing groups can be, for example, straight or branched chain alkyl groups having from 1 to 20 carbon atoms, typically from 1 to 12 carbon atoms, it being possible for the alkyl groups to be further substituted, for example, by at least one halogen atom, such as a fluorine atom or other functional group such as those noted for the R^1 to R^6 groups above. By specifying alkyl groups such as propyl or butyl, it is intended to encompass any isomer of the particular alkyl group.

[0052] In one aspect, examples of alkoxy groups include, but are not limited to, methoxy, ethoxy, propoxy, and butoxy groups, and the like, which can also be further substituted. For example the alkoxy group can be substituted by at least one fluorine atom, with 2,2,2-trifluoroethoxy constituting a useful alkoxy group. In another aspect,

one or more of the alkoxy groups contains at least one fluorine atom. Further, the alkoxy group can contain at least two fluorine atoms or the alkoxy group can contain three fluorine atoms. For example, the polyphosphazene that is combined with the silicone can be poly[bis(2,2,2-trifluoroethoxy)phosphazene]. Alkoxy groups of the polymer can also be combinations of the aforementioned embodiments wherein one or more fluorine atoms are present on the polyphosphazene in combination with other groups or atoms.

[0053] Examples of alkylsulphonyl substituents include, but are not limited to, methylsulphonyl, ethylsulphonyl, propylsulphonyl, and butylsulphonyl groups. Examples of dialkylamino substituents include, but are not limited to, dimethyl-, diethyl-, dipropyl-, and dibutylamino groups. Again, by specifying alkyl groups such as propyl or butyl, it is intended to encompass any isomer of the particular alkyl group.

[0054] Exemplary aryloxy groups include, for example, compounds having one or more aromatic ring systems having at least one oxygen atom, non-oxygenated atom, and/or rings having alkoxy substituents, it being possible for the aryl group to be substituted for example by at least one alkyl or alkoxy substituent defined above. Examples of aryloxy groups include, but are not limited to, phenoxy and naphthoxy groups, and derivatives thereof including, for example, substituted phenoxy and naphthoxy groups.

[0055] The heterocycloalkyl group can be, for example, a ring system which contains from 3 to 10 atoms, at least one ring atom being a nitrogen, oxygen, sulfur, phosphorus, or any combination of these heteroatoms. The hetereocycloalkyl group can be substituted, for example, by at least one alkyl or alkoxy substituent as defined above. Examples of heterocycloalkyl groups include, but are not limited to, piperidinyl, piperazinyl, pyrrolidinyl, and morpholinyl groups, and substituted analogs thereof.

[0056] The heteroaryl group can be, for example, a compound having one or more aromatic ring systems, at least one ring atom being a nitrogen, an oxygen, a sulfur, a phosphorus, or any combination of these heteroatoms. The heteroaryl group can be substituted for example by at least one alkyl or alkoxy substituent defined above. Examples of heteroaryl groups include, but are not limited to, imidazolyl, thiophene,

furane, oxazolyl, pyrrolyl, pyridinyl, pyridinolyl, isoquinolinyl, and quinolinyl groups, and derivatives thereof, such as substituted groups.

[0057] The diameter of a particle formed according to the invention will necessarily vary depending on the end application in which the particle is to be used. The diameter of such particles is preferably about 0.1 μm to about 5,000 μm, with a diameter of about 0.1 μm to about 1,000 μm being most preferred. Other preferred sizes include diameters of about 40 μm, 0.1 to about 10 μm, 100 to about 500 μm, about 1 to about 200 μm and greater than about 500 μm. In methods using the particle where more than one particle is preferred it is not necessary that all particles be of the same diameter or shape.

[0058] The particles may also include other compounds which function to enhance, alter or otherwise modify the behavior of the polymer or particle either during its preparation or in its therapeutic and/or diagnostic use. For example, active agents such as peptides, proteins, hormones, carbohydrates, polysaccharides, nucleic acids, lipids, vitamins, steroids and organic or inorganic drugs may be incorporated into the particle. Excipients such as dextran, other sugars, polyethylene glycol, glucose, and various salts, including, for example, chitosan glutamate, may be included in the particle.

[0059] Additionally, if desired, polymers other than the poly[bis(trifluoroethoxy)phosphazene] and/or its derivative may be included with in the particle. Examples of polymers may include poly(lactic acid), poly(lactic-co-glycolic acid), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyorthoesters, polyacetals, polycyanoacrylates, and polyurethanes. Other polymers include polyacrylates, ethylene-vinyl acetate co-polymers, acyl substituted cellulose acetates and derivatives thereof, degradable or non-degradable polyurethanes, polystyrenes, polyvinylchloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolefins, and polyethylene oxide. Examples of polyacrylates include, but are not limited to, acrylic acid, butyl acrylate, ethylhexyl acrylate, methyl acrylate, ethyl acrylate, acrylonitrile, methyl methacrylate, TMPTA (trimethylolpropane triacrylate), and the like. One may incorporate the selected compounds by any means known in the art, including diffusing, inserting or entrapping the additional compounds in the matrix of an already formed particle or by adding the additional compound to a

polymer melt or to a polymer solvent in the preparation of the particle such as described herein.

[0060] The loaded or unloaded particle may be coated with an additional polymer layer or layers, including polymers such as those mentioned hereinabove. Further, poly[bis(trifluoroethoxy)phosphazene or its derivatives may be used to form such a coating on a particle formed of other suitable polymers or copolymers known or to be developed in the art that are used to form particles as described herein. Preferably, when coating a particle such as a microparticle, poly[bis(trifluoroethoxy)phosphazene is applied as a coating on a microparticle(s) formed of an acrylic-based polymer as set forth in further detail below.

[0061] Coatings are beneficial, for example, if the particle(s) are to be used in a sustained release, orally administered, drug delivery formulation (enteric coating) or if the particles are to be loaded with a potentially toxic contrast agent (non-biodegradable coating).

[0062] The microspheres may be prepared by any means known in the art that is suitable for the preparation of particles containing poly[bis(trifluoroethoxy)phosphazene]. In a procedure according to an embodiment herein a "polymer solution" is prepared by mixing one or more polymer solvent(s) and the poly[bis(trifluoroethoxy)phosphazene] and/or a derivative thereof until the polymer is dissolved.

[0063] Suitable solvents for use in the preparation of the polymer solution include any in which the polymer poly[bis(trifluoroethoxy)phosphazene] and/or its derivatives are soluble. Exemplary solvents include, without limitation, ethyl-, propyl-, butyl-, pentyl-, octylacetate, acetone, methylethylketone, methylpropylketone, methylisobutylketone, tetrahydrofurane, cyclohexanone, dimethylacetamide, acetonitrile, dimethyl ether, hexafluorobenzene or combinations thereof.

[0064] The polymer solution contains the poly[bis(trifluoroethoxy)phosphazene] and/or its derivative polymer in a concentration of about 1% by weight of polymer to 20% by weight of polymer, preferably about 5% to 10% by weight of polymer. Other polymers, as discussed above, may be present in the solution, or may be added to the vessel in the

form of a second solution powder or other form, if one wishes to include such polymers in the final particle.

[0065] In carrying out the process, the polymer solution is next dispensed, preferably in the form of drops or an aerosol, into a vessel containing a non-solvent. By "non-solvent" it is meant any organic or inorganic solvents that do not substantially dissolve the poly[bis(trifluoroethoxy)phosphazene] polymer and which have a melting point that is lower relative to the melting point of the solvent in which the polymer is dissolved ("polymer solvent"), so that the non-solvent thaws before the solvent thaws in the course of the incubation step. Preferably, this difference between the melting point of the non-solvent and the polymer solvent is about 10° C, more preferably about 15° C, and most preferably, greater than about 20° C. Under certain conditions it has been found that the structural integrity of the resultant particle may be enhanced if the difference of the melting points of the polymer solvent and of the non-solvent is greater than 15° C. However, it is sufficient that the non-solvent point is merely slightly lower than that of the polymer solvent.

[0066] The non-solvent/polymer solvent combination is incubated for approximately 1 to 5 days or until the polymer solvent has been completely removed from the particles. While not wishing to be bound by theory, it is hypothesized that during the incubation, the non-solvent functions to extract the polymer solvent from the microscopic polymer solution droplets from the particles such that the polymer is at least gelled. As the incubation period passes, the droplets will shrink and the solvent becomes further extracted, leading to a hardened outer polymeric shell containing a gelled polymer core, and finally, after completion of the incubation, a complete removal of the residual solvent. To ensure that the polymeric droplets retain a substantially spherical shape during the incubation period, they are maintained in a frozen or substantially gelled state during most if not all of the incubation period. Therefore, the non-solvent temperature may stay below the melting point of the solvent during the cryoextraction process.

[0067] As shown in Fig. 1, at the vessel labeled (a), polymer solution droplets are shown being dispensed either with a syringe or other device at a controlled rate onto a top layer of liquid nitrogen. The nitrogen layer is situated over a bottom layer consisting of the selected non-solvent, which will eventually serve to extract the solvent from the frozen

polymer solution droplets. The non-solvent layer has been previously frozen with liquid nitrogen prior to the dispensing of the polymer solution. The vessel labeled (b) shows the onset of the dewing of the frozen nonsolvent, into which the frozen polymeric droplets will sink. The vessel labeled (c) shows the cryoextraction procedure after approximately three days of incubation wherein the polymer solution droplets, incubated within the non-solvent, have been depleted of a substantial amount of solvent. The result is a gelled, polymeric particle in the form of a bead having a hardened outer shell. As can be seen by the representation, the non-solvent height within the vessel is slightly reduced due to some evaporation of the non-solvent. The size of the beads will shrink quite substantially during this process depending on the initial concentration of the polymer in the polymer solution.

[0068] In one embodiment of a method of preparing a poly[bis(trifluoroethoxy)phosphazene]-containing particle(s) according to the invention, such particles can be formed using any way known or to be developed in the art. Two exemplary preferred methods of accomplishing this include wherein (i) the non-solvent residing in the vessel in the method embodiment described above is cooled to close to its freezing point or to its freezing point prior to the addition of the polymer solution such that the polymer droplets freeze upon contact with the pre-cooled non-solvent; or (ii) the polymer droplets are frozen by contacting them with a liquefied gas such as nitrogen, which is placed over a bed of pre-frozen non-solvent (see, Fig. 2). In method (ii), after the nitrogen evaporates, the non-solvent slowly thaws and the microspheres in their frozen state will sink into the liquid, cold non-solvent where the extraction process (removal of the polymer solvent) will be carried out.

[0069] By modifying this general process, one may prepare particles that are hollow or substantially hollow or porous. For example, if the removal of the solvent from the bead is carried out quickly, *e.g.*, by applying a vacuum during the final stage of incubation, porous beads will result.

[0070] The particles of the invention can be prepared in any size desired, "Microspheres" may be obtained by nebulizing the polymer solution into a polymer aerosol using either pneumatic or ultrasonic nozzles, such as, for example a Sonotek 8700-60ms or a Lechler US50 ultrasonic nozzle, each available from Sono[.tek] Corporation, Milton, New York, U.S.A. and Lechler GmbH, Metzingen, Germany. Larger particles

may be obtained by dispensing the droplets into the non-solvent solution using a syringe or other drop-forming device. Moreover, as will be known to a person of skill in the art, the size of the particle may also be altered or modified by an increase or decrease of the initial concentration of the polymer in the polymer solution, as a higher concentration will lead to an increased sphere diameter.

[0071] In an alternative embodiment of the particles described herein, the particles can include a standard and/or a preferred core based on an acrylic polymer or copolymer with a shell of poly[bis(trifluoroethoxy)phosphazene]. The acrylic polymer based polymers with poly[bis(trifluoroethoxy)phosphazene] shell described herein provide a substantially spherical shape, mechanical flexibility and compressibility, improved specific gravity properties. The core polymers may be formed using any acceptable technique known in the art, such as that described in B. Thanoo et al., "Preparation of Hydrogel Beads from Crosslinked Poly(Methyl Methacrylate) Microspheres by Alkaline Hydrolysis," J. Appl. P. Sci., Vol. 38, 1153-1161 (1990), incorporated herein by reference with respect thereto. Such acrylic-based polymers are preferably formed by polymerizing unhydrolyzed precursors, including, without limitation, methyl acrylate (MA), methyl methacrylate (MMA), ethylmethacrylate (EMA), hexamethyl (HMMA) or hydroxyethyl methacrylate (HEMA), and derivatives, variants or copolymers of such acrylic acid derivatives. Most preferred is MMA. The polymer should be present in the core in a hydrated or partially hydrated (hydrogel) form. Such polymers are preferably cross-linked in order to provide suitable hydrogel properties and structure, such as enhanced non-biodegradability, and to help retain the mechanical stability of the polymer structure by resisting dissolution by water.

[0072] Preferably, the core prepolymers are formed by dispersion polymerization that may be of the suspension or emulsion polymerization type. Emulsion polymerization results in substantially spherical particles of about 10 nm to about 10 microns. Suspension polymerization results in similar particles but of larger sizes of about 50 to about 1200 microns.

[0073] Suspension polymerization may be initiated with a thermal initiator, which may be solubilized in the aqueous or, more preferably, monomer phase. Suitable initiators for use in the monomer phase composition include benzoyl peroxide, lauroyl peroxide or other

similar peroxide-based initiators known or to be developed in the art, with the most preferred initiator being lauroyl peroxide. The initiator is preferably present in an amount of about 0.1 to about 5 percent by weight based on the weight of the monomer, more preferably about 0.3 to about 1 percent by weight based on the weight of the monomer. As noted above, a cross-linking co-monomer is preferred for use in forming the hydrated polymer. Suitable cross-linking co-monomers for use with the acrylic-based principle monomer(s) used in preparing a polymerized particle core, include various glycol-based materials such as ethylene glycol dimethacrylate (EGDMA), diethylene glycol dimethacrylate (DEGDMA) or most preferably, triethylene glycol dimethacrylate (TEGMDA). A chain transfer agent may also be provided if desired. Any suitable MA polymerization chain transfer agent may be used. In the preferred embodiment herein, dodecylmercaptane may be used as a chain transfer agent in amounts acceptable for the particular polymerization reaction.

[0074] The aqueous phase composition preferably includes a surfactant/dispersant as well as a complexing agent, and an optional buffer is necessary. Surfactants/dispersants should be compatible with the monomers used herein, including Cyanamer® 370M, polyacrylic acid and partially hydrolyzed polyvinyl alcohol surfactants such as 4/88, 26/88, 40/88. A dispersant should be present in an amount of about 0.1 to about 5 percent by weight based on the amount of water in the dispersion, more preferably about 0.2 to about 1 percent by weight based on the amount of water in the dispersion. An optional buffer solution may be used if needed to maintain adequate pH. A preferred buffer solution includes sodium phosphates (Na_2HPO_4/NaH_2PO_4). A suitable complexing agent is ethylene diamine tetraacetic acid (EDTA), which may be added to the aqueous phase in a concentration of from about 10 to about 40 ppm EDTA, and more preferably about 20 to about 30 ppm. It is preferred that in the aqueous phase composition, the monomer to water ratio is about 1:4 to about 1:6.

[0075] The polymerization should take place at about ambient conditions, preferably from about 60° C to about 80° C with a time to gelation of about one to two hours. Stirring at rates of 100 to 500 rpm is preferred for particle formation, with lower rates applying to larger sized particles and higher rates applying to smaller sized particles.

[0076] Once PMMA particles, such as microparticles, are formed, they are preferably subjected to hydrolysis conditions typical of those in the art, including use of about 1-10 molar excess of potassium hydroxide per mol of PMMA. Such potassium hydroxide is provided in a concentration of about 1-15% potassium hydroxide in ethylene glycol. The solution is then heated preferably at temperatures of about 150-185° C for several hours. Alternatively, to minimize reactant amounts and cost, it is preferred that lesser amounts of potassium hydroxide be used which are less than about 5 molar excess of potassium hydroxide per mole of PMMA, more preferably about 3 molar excess or less. For such hydrolytic reactions, a concentration of about 10-15% potassium hydroxide in ethylene glycol is also preferably used, and more preferably about 14% to about 15%. It will be understood by one skilled in the art, that heating conditions at higher temperatures may be used to decrease overall reaction times. Reaction times may be varied depending on the overall diameter of the resultant particles. For example, the following conditions are able to provide particles having about 35% compressibility and desired stability: for diameters of about 200-300 μm, the solution should be heated for about 7.5 to about 8.5 hours; for diameters of about 300-355 μm, about 9.5 to about 10.5 hours; for diameters of about 355-400 μm, about 11.5 to about 12.5 hours; and for about 400-455 μm, about 13.5 to about 14.5 hours, etc. The particle size can be adjusted using variations in the polymerization process, for example, by varying the stirring speed and the ratio of the monomer to the aqueous phase. Further, smaller sizes can be achieved by increasing surfactant/dispersant ratio.

[0077] Following hydrolysis, particles are separated from the reaction mixture and their pH may be adjusted to any range as suited for further processing steps or intended uses. The pH of the particle core may be adjusted in from about 1.0 to about 9.4, preferably about 7.4 if intended for a physiological application. Since size, swelling ratio and elasticity of the hydrogel core material are dependent on pH value, the lower pH values may be used to have beneficial effects during drying to prevent particle agglomeration and/or structural damage. Particles are preferably sieved into different size fractions according to intended use. Drying of particles preferably occurs using any standard drying process, including use of an oven at a temperature of about 40° -80° C for several hours up to about a day.

[0078] To provide desired surface properties to the hydrophilic hydrogel particles, in order to provide adhesion for receiving a poly[bis(trifluoroethoxy)phosphazene] coating, the surface of the hydrogel may be subjected to treatment with any suitable ionic or non-ionic surfactant, such as tetraalkylammonium salts, polyalcohols and similar materials. A more permanent change in adhesion properties is brought about by rendering the surface of the particles hydrophobic by reaction of its polymethacrylic acid groups with a suitable reactant. Suitable reactants include, but are not limited to, hydrophobic alcohols, amides and carboxylic acid derivatives, more preferably they include halogenated alcohols such as trifluoroethanol. Such surface treatment also prevents delamination of the coating from the core once the coating is applied. Preferred surface treatments may include, without limitation, an initial treatment with thionyl chloride followed by reaction with trifluoroethanol. Alternatively, the surface may be treated by suspending the particles in a mixture of sulfuric acid and a hydrophobic alcohol, such as trifluoroethanol. Such treatments are preferred if the particles are to be coated in that they minimize any delamination of a coating.

[0079] Alternatively, in some preferred embodiments of the present invention, the PMA core particles may be coated with a surface layer of and/or infused with barium sulfate. The barium sulfate is radiopaque and aids in visualization of the finished particles when in use. It also provides enhanced fluidization properties to the particles such that it reduces agglomeration especially during drying and allows for fluid bed coating of the PMA particles with an outer coating of poly[bis(trifluoroethoxy)phosphazene], thereby providing improved adhesion between a poly[bis(trifluoroethoxy)phosphazene] outer core and a polymeric acrylate core particles. By allowing fluidization even when the core particles are swollen, barium sulfate also improves the overall coating and adhesion properties. By enabling the coating of the core particles even in a swollen state with poly[bis(trifluoroethoxy)phosphazene], barium sulfate also reduces the potential tendency of the poly[bis(trifluoroethoxy)phosphazene] shells to crack or rupture in comparison with coating the particles in a dry state and then later exposing the particles to a suspension in which the core particles swell and exert force on the shell of poly[bis(trifluoroethoxy)phosphazene]. A coating of barium sulfate on the core particles is preferably applied by adhesion of the barium sulfate in the form of an opaque coating on the hydrogel surface of the PMA beads. Barium sulfate can further assist in reducing

electrostatic effects that limit particle size. By allowing for absorption of additional humidity, the barium sulfate tends to counteract the electrostatic effects.

[0080] Barium sulfate crystals adhering only loosely to the PMA particles may be covalently crosslinked or chemically grafted to the particle surface by spraycoating a sufficient amount of an aminosilane adhesion promoter onto the PMA particle. This will help to effectively reduce barium sulfate particulate matter in solution after hydration of the particles. Exemplary particles include 3-aminopropyl-trimethoxysilane and similar silane-based adhesion promoters.

[0081] A further alternative for improving visualization of and potential functionality of microparticles made as noted herein include the absorption of a water soluble organic dye inside the hydrogel core particles. Exemplary dyes are preferably those FDA dyes approved for human use and which are known or to be developed for safe, non-toxic use in the body and which are capable of providing acceptable contrast. Organic dyes may include dyes such as D&C Violet no. 2 and others preferably approved for medical device uses, such as for contact lenses and resorbable sutures. Whereas barium sulfate operates as an inorganic filler and finely dispersed pigment that makes the particles visible by light diffraction due to small crystal size, the dyes when impregnated in the particles absorb the complementary part of the visible color spectrum.

[0082] Water soluble organic dyes in various embodiments of the present invention may be provided in colors that approximate various shades of human flesh or other tissue tones for improved cosmesis.

[0083] Yet another alternative embodiment of the present invention relates to the use of custom color dyes for inclusion in the microspheres for patient-specific applications. These applications include, but are not limited to, situations in which such microspheres are to be introduced and left within thin or superficial tissue, where the presence of the microspheres might otherwise be visible to an observer. In such embodiments, a user would first provide a quantitative analysis of a desired tissue using a hand-held spectrophotometer or other device to records data from a desired area of a mammalian patient's skin is used in conjunction with a computerized color formulation system. Based on this color measurement, a color formula will be calculated by the computer, and

appropriate dyes will be mixed to produce pigmented microspheres to match the color of the desired target skin.

[0084] Particles, including microparticles made in accordance with the foregoing process for forming a core hydrogel polymer are then coated with poly[bis(trifluoroethoxy)phosphazene] and/or its derivatives. Any suitable coating process may be used, including solvent fluidized bed and/or spraying techniques. However, preferred results may be achieved using fluidized bed techniques in which the particles pass through an air stream and are coated through spraying while they spin within the air stream. The poly[bis(trifluoroethoxy)phosphazene] or derivative polymer is provided in dilute solution for spraying to avoid clogging of the nozzle.

[0085] Exemplary solvents for use in such solutions include ethyl acetate, acetone, hexafluorbenzene, methyl ethyl ketone and similar solvents and mixtures and combinations thereof, most preferred is ethyl acetate alone or in combination with isoamyl acetate. Typical preferred concentrations include about 0.01 to about 0.3 weight percent poly[bis(trifluoroethoxy)phosphazene] or its derivative in solution, more preferably about 0.02 to 0.2 weight percent poly[bis(trifluoroethoxy) phosphazene], and most preferably about 0.075 to about 0.2 weight percent. It should be understood based on this disclosure that the type of hydrogel core can be varied as can the technique for coating a particle, however it is preferred that a core which is useful in the treatment techniques and applications described herein is formed and subsequently coated with poly[bis(trifluoroethoxy)phosphazene] and/or its derivatives as described herein.

[0086] One method for increasing the density of the particles is by use of heavy water or deuterium oxide (D_2O). When heavy water is used to swell the particles, D_2O displaces H_2O, thereby increasing the weight of the particles for better dispersion and buoyancy levels. Typically this leads to the ability to add higher amounts of contrast agent of at least about 5% using such a technique. However, some equilibrating effect can occur over time when the particles are contacted with an aqueous solution of contrasting agent. Thus, it is preferred that when using D_2O for this purpose, either that suspension times are kept to a minimum or, more preferably, that the contrast agent be provided in a solution which also uses D_2O.

[0087] Alternatively, particles of pH 1 can be neutralized with cesium hydroxide and/or the final neutralized particles can be equilibrated with cesium chloride. Such compounds diffuse cesium into the particles, such that either the cesium salt of polymethacrylic acid is formed or polymethacrylic acid is diffused and thereby enriched with cesium chloride.

[0088] The cesium increases the density of the particles, thereby increasing the ability to add higher amounts of contrast agent. Typical buoyancy levels can be adjusted using the cesium technique such that about 45 to about 50% contrast agent may be added to the delivery medium as is desired for embolization. Cesium salts are non-toxic and render the particles visible using fluoroscopy. Cesium's atomic weight of 132.9 g/mol is slightly higher than that of iodine providing beneficial effects including increase in overall density and enhancement of X-ray contrast visibility even without a contrast agent. For certain cancer treatments where a radioactive isotope of cesium is desired, such active agent can be used as an alternative cesium source rendering the particles buoyant in an embolic solution as well as able to be used as an active treatment source.

[0089] The above-noted techniques for improving density of particles, such as microparticles for embolization or other applications where density and/or buoyancy in solution are applicable properties may be applied in to the preferred particles described herein and/or may be applied for other similar particles. It should be understood that the disclosure is not limited to cesium and/or D_2O treatment of the preferred particles herein and that such techniques may have broader implications in other particles such as other acrylic-based hydrogels and other polymeric particles.

[0090] As noted above, barium sulfate may be used between the core particles and the preferred poly[bis(trifluoroethoxy)phosphazene] coating or introduced into the interior of the core particles using any technique known or to be developed in the art. Also, organic dyes may similarly be included in the particle core. These materials, particularly the barium sulfate, also contribute to an increase in density as well as providing radiopacity. In addition to a general density increase as provided by the above-noted D_2O or cesium compounds, the barium sulfate allows this benefit even upon substantial and/or full hydration, allowing particles in suspension to remain isotonic. Thus, a barium sulfate

powder coating can provide an inert precipitate having no effect on physiological osmolarity.

[0091] It should be understood, based on this disclosure, that the various buoyancy additives noted above can be used independently or in combination to provide the most beneficial effects for a given core particle and coating combination.

[0092] The invention also includes methods of delivering an active agent to a localized area within the body of a mammal. The method includes contacting the localized area with at least one of the particles of the invention as described above, such that an effective amount of the active agent is released locally to the area. Diseases or pathologies that may be treated by this method include any wherein the localized or topical application of the active agent achieves some benefit in contrast to the systemic absorption of the drug. Suitable active agents include NSAIDS, steroids, hormones, and nucleic acids,

[0093] If the particle formulated for delivery of an active agent to a localized area is about 1 to about 1,000 μm in diameter, the drug loaded microspheres can be applied to localized areas within the mammalian body using syringes and/or catheters as a delivery device, without causing inadvertent occlusions. For example, using a contrast agent, a catheter can be inserted into the groin artery and its movement monitored until it has reached the area where the localized administration is desired. A dispersion of the particles in a suitable injection medium can be injected through the catheter, guaranteeing only a specific area of the body will be subjected to treatment with drug loaded beads (particles). As will be understood to a person of skill in the art, injection mediums include any pharmaceutically acceptable mediums that are known or to be developed in the art, such as, *e.g.*, saline, PBS or any other suitable physiological medium. In accordance with a further embodiment described herein, the invention may include an injectable dispersion including particles and a contrasting agent which particles are substantially dispersed in the solution. In a preferred embodiment, the particles may also detectible through fluoroscopy or other imaging modalities.

[0094] The polymeric particles of the invention may be used to prepare a sustained release formulation of an active agent for local administration. The formulation comprises a particle, as described above, loaded with an active agent. The polymeric particle utilized may be hollow, substantially hollow or solid. The particle can be loaded with the active

agent either by dispersion or solvation of the active agent in the polymer solution prior to the production of micro-sized particles through spray droplets, pastillation of a polymer melt or carrying out of a cryoextraction process. Alternatively, an unloaded polymer particle can be prepared and subsequently immersed in solutions containing active agents. The particles are then incubated in these solutions for a sufficient amount of time for the active agent to diffuse into the matrix of the polymer. After drying the particles, the active agent will be retained in the polymer particle. If this loading mechanism is utilized, drug loading can be controlled by adjusting drug concentrations of the incubation medium and removing the particles from the incubation medium when an equilibrium condition has been attained.

[0095] The present invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort can be had to various other aspects, embodiments, modifications, and equivalents thereof which, after reading the description herein, can suggest themselves to one of ordinary skill in the art without departing from the spirit of the present invention or the scope of the appended claims.

[0096] Further, it is to be understood that this invention is not limited to specific materials, agents, polyphosphazenes, or other compounds used and disclosed in the invention described herein, including in the following examples, as each of these can vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects or embodiments and is not intended to be limiting. Should the usage or terminology used in any reference that is incorporated by reference conflict with the usage or terminology used in this disclosure, the usage and terminology of this disclosure controls.

[0097] Unless indicated otherwise, temperature is reported in degrees Centigrade and pressure is at or near atmospheric. An example of the preparation of a polyphosphazene of this invention is provided with the synthesis of poly[bis(trifluoroethoxy)phosphazene] polymer, which may be prepared according to U.S. Patent Application Publication No. 2003/0157142, the entirety of which is hereby incorporated by reference.

[0098] Also unless indicated otherwise, when a range of any type is disclosed or claimed, for example a range of molecular weights, layer thicknesses, concentrations, temperatures, and the like, it is intended to disclose or claim individually each possible number that such a range could reasonably encompass, including any sub-ranges encompassed therein. For example, when the Applicants disclose or claim a chemical moiety having a certain number of atoms, for example carbon atoms, Applicants' intent is to disclose or claim individually every possible number that such a range could encompass, consistent with the disclosure herein. Thus, by the disclosure that an alkyl substituent or group can have from 1 to 20 carbon atoms, Applicants intent is to recite that the alkyl group have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. In another example, by the disclosure that microspheres have a diameter of approximately 500 to 600 μm, Applicants include within this disclosure the recitation that the microspheres have a diameter of approximately 500 μm, approximately 510 μm, approximately 520 μm, approximately 530 μm, approximately 540 μm, approximately 550 μm, approximately 560 μm, approximately 570 μm, approximately 580 μm, approximately 590 μm, and/or approximately 600 μm, including any range or sub-range encompassed therein. Accordingly, Applicants reserve the right to proviso out or exclude any individual members of such a group, including any sub-ranges or combinations of sub-ranges within the group, that can be claimed according to a range or in any similar manner, if for any reason Applicants choose to claim less than the full measure of the disclosure, for example, to account for a reference that Applicants are unaware of at the time of the filing of the application.

EXAMPLE 1

[0099] Microspheres having a diameter of approximately 500 to 600 μm were prepared. First, a polymer solution was prepared by dissolving poly[bis(trifluoroethoxy)phosphazene] polymer of a molecular weight $3x10^6$ g/mol in the polymer solvent ethyl acetate to obtain a 2% (wt/v) polymer solution. Four milliliters of this polymer solution was manually dripped into liquid nitrogen using a 5 ml syringe. This dispersion was dispensed onto a frozen layer of 150 milliliters of pentane. (See Fig. 2.) The cryoextraction was allowed to proceed for three days. Subsequently, polymeric particles were retrieved from the reaction vessel, and were air dried at 21° C.

EXAMPLE 2

[00100] Microspheres having a diameter of approximately 350 to 450 μm were prepared. First, a polymer solution was prepared by dissolving poly[bis(trifluoroethoxy)phosphazene] polymer of a molecular weight $3x10^6$ g/mol in ethyl acetate to obtain a 1% (wt/v) polymer solution. Four milliliters of this polymer solution was manually dripped into liquid nitrogen using a 5 ml syringe. This dispersion was dispensed onto a frozen layer of 150 milliliters of pentane. (See Fig. 2.) The cryoextraction was allowed to proceed for three days. Subsequently, polymeric particles were retrieved from the reaction vessel and were air dried at 21° C.

EXAMPLE 3

[00101] Microspheres having a diameter of approximately 500 to 600 μm were prepared. First, a polymer solution was prepared by dissolving poly[bis(trifluoroethoxy)phosphazene] polymer of a molecular weight $12x10^6$ g/mol in methylisobutylketone to obtain a 2% (wt/v) polymer solution. Four milliliters of this polymer solution was manually dripped into liquid nitrogen using a 5 ml syringe. This dispersion was dispensed onto a frozen layer of 150 milliliters of a 1:9 (v/v) ethanol/pentane mixture (See Fig. 2.). The cryoextraction was allowed to proceed for three days. Subsequently, polymeric particles were retrieved from the reaction vessel, and dried under reduced pressure at 21° C.

EXAMPLE 4

[00102] Microspheres having a diameter of approximately 500 to 600 μm were prepared. First, a polymer solution was prepared by dissolving poly[bis(trifluoroethoxy)phosphazene] polymer of a molecular weight $9x10^6$ g/mol in isoamylketone to obtain a 2% (wt/v) polymer solution. Four milliliters of this polymer solution was manually dripped into liquid nitrogen using a 5 ml syringe. This dispersion was dispensed onto a frozen layer of 150 milliliters of pentane. (See Fig. 2.) The cryoextraction was allowed to proceed for three days. Subsequently, polymeric polymers were retrieved from the reaction vessel and dried under reduced pressure at 21° C.

EXAMPLE 5

[00103] Microspheres having a diameter of approximately 500 to 600 μm were prepared. First, a polymer solution was prepared by dissolving poly[bis(trifluoroethoxy)phosphazene] polymer of a molecular weight $16x10^6$ g/mol in cyclohexanone to obtain a 2% (wt/v) polymer solution. Four milliliters of this polymer solution was manually dropped into liquid nitrogen using a 5 ml syringe. This dispersion was dispensed onto a frozen layer of 150 milliliters of a 1:1 (v/v) ethanol/diethyl ether mixture. (See Fig. 2.) The cryoextraction was allowed to proceed for three days. Subsequently, polymeric particles were retrieved from the reaction vessel and dried under reduced pressure at 21° C.

EXAMPLE 6

[00104] Microspheres having a diameter of approximately 500 to 600 μm were prepared. First, a polymer solution was prepared by dissolving poly[bis(trifluoroethoxy)phosphazene] polymer of a molecular weight $3x10^6$ g/mol in ethyl acetate to obtain a 2% (wt/v) polymer solution. Four milliliters of this polymer solution was manually dripped into liquid nitrogen using a 5 ml syringe. This dispersion was dispensed onto a frozen layer of 150 milliliters of hexane. (See Fig. 2.) The cryoextraction was allowed to proceed for three days. Subsequently, polymeric particles were retrieved from the reaction vessel and air dried at 21° C.

EXAMPLE 7

[00105] Microspheres having a diameter of approximately 500 to 600 μm were prepared. First, a polymer solution was prepared by dissolving poly[bis(trifluoroethoxy)phosphazene] polymer of a molecular weight $3x10^6$ g/mol in ethyl acetate to obtain a 2% (wt/v) polymer solution. Four milliliters of this polymer solution was manually dripped into liquid nitrogen using a 5 ml syringe. This dispersion was dispensed onto a frozen layer of 150 milliliters of ethanol. (See Fig. 2.) The cryoextraction was allowed to proceed for three days. Subsequently, polymeric particles were retrieved from the reaction vessel and air dried at 21° C. The particles were noticeably gel-like and after drying were ellipsoid in shape.

EXAMPLE 8

[00106] Microspheres having a diameter of approximately 500 to 600 μm were prepared. First, a polymer solution was prepared by dissolving poly[bis(trifluoroethoxy)phosphazene] polymer of a molecular weight $3x10^6$ g/mol in ethyl acetate to obtain a 2% (wt/v) polymer solution. Four milliliters of this polymer solution was manually dripped into liquid nitrogen using a 5 ml syringe. This dispersion was dispensed onto a frozen layer of 150 milliliters of diethylether. (See Fig. 2.) The cryoextraction was allowed to proceed for three days. Subsequently, polymeric particles were retrieved from the reaction vessel and air dried at 21° C. The resultant particles were, after drying, compact and uniformly spherical.

EXAMPLE 9

[00107] A two liter cryovessel as shown in Fig. 6 was filled with 100 milliliters of diethyl ether as a non-solvent. Liquid nitrogen was slowly added until the non-solvent froze. The vessel was then filled with additional liquid nitrogen, until the amount of liquid nitrogen rose approximately 5 to 10 cm when measured vertically above the non-solvent layer. The vessel was closed with an insulated lid, and a syringe needle connected via Teflon tubing to a syringe pump was inserted through a small opening in the lid.

[00108] The syringe pump as shown in Fig. 7, was used to dispense between 5 to 15 milliliters of the 5 to 40 mg/ml polymer solution in ethyl acetate, slowly into the cryovessel. The rate of the pump was adjusted to approximately 10 milliliters dispensing volume per hour. A Teflon® cylinder with one inlet and one to eight outlets is used to distribute the dispensed volumes into several vessels in parallel. (It is preferable that the ratio of solvent to non-solvent volume stays below 10% (v/v). Otherwise the particles may adhere to one another.) After the polymer solution was completely dispensed into the vessel, another 100 milliliters of non-solvent was slowly poured on top of the liquid nitrogen.

[00109] In carrying out this process, it is noted that it is preferable that the needle tips used for dispensing are small, such as the G33 size. Additionally, the dropping distance should be more than 5 cm, so that the droplets aided by gravity immediately sink into the liquid nitrogen upon hitting the surface.

[00110] The liquid nitrogen in the vessel was slowly allowed to evaporate, taking approximately one day. The non-solvent slowly began to melt, and the polymer solution droplets, still frozen, sank into the cold non-solvent. After another day of incubation, the now gelled polymer beads (particles) were retrieved from the vessel by simple filtration. They were allowed to dry at room temperature for approximately 30 minutes and then were ready for use in any of the applications described herein.

EXAMPLE 10

[00111] The microspheres prepared by the process of Example 1 were examined for shape and surface morphology by optical microscope, scanning electron microscope (SEM) and atomic force microscopy. The results of these analyses are shown in Figs. 3A and 3B). Fig. 3A shows the microspheres as they appear using an optical microscope at 4x magnification. Fig. 3B shows a microsphere as it appears using a scanning electron microscope at 100x magnification.

[00112] It can be seen that surface morphology of the unloaded spheres is typical for semi-crystalline polymers above glass transition temperature. Amorphous as well crystalline regions are prevalent throughout the sample surface. The surface is microporous in nature, with pore sizes ranging from nanometers to few micrometers in diameter.

[00113] Particles loaded with bovine insulin were also analyzed using scanning electron microscopy (100x magnification). The result of these analyses can be seen in Figs. 4A and Fig. 4B).

EXAMPLE 11

[00114] Several polymerizations were carried out using varying combinations of PMMA and three different crosslinking monomers (EDGMA, DEGDMA and TEGDMA), different radical initiators (benzoyl peroxide (BPO) and lauroyl peroxide (LPO), EDTA as a complexing agent and varying dispersants (Cyanamer 370M, polyacrylic acid (PAA) and varying types of polyvinyl alcohol (PVA) to achieve the preferred core particles. In some polymerizations, sodium phosphate buffer solution (Na_2HPO_4/NaH_2PO_4) was used. It was observed that some of the reaction procedures went unsuccessful due to the type of dispersant and concentration chosen. Failure of the dispersant was demonstrated in the

form of early onset of an exothermic reaction, coalescing aqueous and organic phases and premature onset of the vitrification phase. Only the successful examples are shown. The successful runs are shown below in Table 1, which includes the components, concentrations and reaction conditions for such samples (1-6).

TABLE 1

Sample	1	2	3	4	5	6
Monomer	PMMA 99.0 g	PMMA 190.0 g	PMMA 182.0 g	PMMA 200.2 g	PMMA 200.2g	PMMA 200.2 g
Crosslinker	EGDMA (1 wt%/ monomer)	EGDMA (1 wt%/ monomer)	EGDMA (1 wt%/ monomer)	DEGDMA (0.5 mol%/ monomer)	TEGDMA (0.5 mol%/ monomer)	TEGDMA (0.5 mol%/ monomer 7.5 mMol DDM)
Radical Initiator	LPO (0.3 wt% monomer	LPO (0.3 wt% monomer)	LPO (0.3 wt% monomer)	LPO (0.3 wt% monomer)	LPO (0.3 wt% monomer)	LPO (0.3 wt% monomer)
Complexing Agent	EDTA 22 mg	EDTA 44 mg	EDTA 44 mg	EDTA 56 mg	EDTA 56 mg	EDTA 56 mg
Monomer/ Water Ratio	1:5	1:5	1:5	1:6	1:6	1:6
Dispersant	PVA 4/88 35% PVA 26/88 65% 1 wt% / water	PVA 4/88 35% PVA 26/88 65% 0.5 wt%/ water	PVA 26/88 0.25 wt%/ water	PVA 26/88 0.23 wt%/ water	PVA 26/88 0.23 wt%/ water	PVA 26/88 0.23 wt%/ water
Buffer Solution	No	No	No	Yes	Yes	Yes
Reaction Temperature/Time	1h 67° C 2 h 70° C 1 h 80° C	1h 67° C 2 h 70° C 1 h 80° C	1h 67° C 2 h 70° C 1 h 80° C	1h 67° C 2 h 70° C 1 h 80° C	1h 67° C 2 h 70° C 1 h 80° C	1h 67° C 2 h 70° C 1 h 80° C
Outcome (particle size)	1-50 µm due to dispersant conc.	20-200 µm due to dispersant conc.	100-200µm due to dispersant conc.	1-100 µm due to initial stirring at 400 rpm	1-100 µm due to initial stirring at 400 rpm	50-1,000 µm due to initial stirring at 130 rpm

EXAMPLE 12

[00115] Hydrogel microparticles formed in accordance with the procedures described herein were evaluated for buoyancy and suspension properties for use in embolization applications. The microparticles included a sample using unmodified polymethacrylic acid potassium salt hydrogel particles (Sample A); a sample using trifluoroethyl esterified polymethacrylic acid potassium salt hydrogels (Sample B); and a sample using the same hydrogel as Sample B, but wherein the particles were coated with poly[bis(trifluoroethoxy)phosphazene] (Sample C). An isotonic phosphate buffered saline solution of pH 7.4 having 0.05 volume % Tween™ 20 was prepared by dissolving 5 phosphate buffered saline tablets (Fluka®) in 999.5 ml of milliQ ultrapure water. 0.5 ml of Tween 20™ surfactant was added to the solution. Solutions having between 20 and 50

percent by volume of Imeron300® contrast agent in the isotonic buffered saline solution were then prepared for evaluation.

[00116] The contrast agent solutions which were prepared were then placed in 4 ml vials in aliquots of 2 ml each. To the vials, 50-80 mg of the hydrated hydrogel Samples A-C were added. Each Sample was first hydrated by adding to 100 mg of dry hydrogel microparticles either 900 mg of isotonic phosphate buffered saline solution or D_2O to obtain 1 ml swollen hydrogel. Buoyancy properties were measured immediately and every 10 minutes thereafter until buoyancy equilibrium was achieved and/or surpassed.

[00117] All of the particles reached equilibrium density in the contrast agent solution having 30-40% contrasting agent within 5 min. Particles which were swollen with D_2O were heavier within the first 10 minutes, but the D_2O did diffuse out of the particles over time within 15-20 min. of immersion. If additional water which could displace the D_2O were not added, microparticles hydrated with D_2O would be able to increase the contrast agent percentage achievable with adequate buoyancy by as much as 5%. Particles began to float to the top over time when the contrast agent was added in percentages of 40%-50%.

[00118] The equilibrium buoyancy (matching densities) was achieved for Sample C in 31 ± 1 volume percent of contrast agent in solution. With regard to Samples A and B, swelling behavior and subsequent density are typically dependent on crosslinking content, pH, ionic strength and valence of cations used. However, it was assumed herein that the swelling does not influence buoyancy due to the sponge-like nature of the polymethacrylic acid hydrogel material. After such material was coated with the poly[bis(trifluoroethoxy)phosphazene] as in Sample C, a time lag of swelling was observed and buoyancy equilibrium was slower to achieve.

EXAMPLE 13

[00119] In order to take account of the time lag and to achieve a more preferred density, as well as to enhance the fluoroscopic visibility of the particles, cesium treatment was then effected for the types of microparticles used in Samples B and C of Example 12.

[00120] 100 mg of Sample C and of Sample B were hydrated each for 10 min. in a 30 weight percent solution of sodium chloride. The supernatant liquid was decanted after equilibrium and the microparticles were washed thoroughly with deionized water. They

were then equilibrated for another 10 min., decanted and suspended in 3 ml of surfactant-free isotonic phosphate buffer solution at a pH 7.4. The effect on buoyancy was then evaluated using contrast agent solutions varying from 20 to 50% by volume of Imeron® 300. In this Example, 0.1 g of the microparticles of Samples B and C were used. 3.5 ml of Imeron 300 contrast agent were provided to the initial buffer solution which included 4.0 ml isotonic phosphate buffer/Tween™ 20 solution.

[00121] The equilibration procedure using cesium chloride yielded particles of increased density. Both microparticle samples showed a final buoyancy in the Imeron® 300 contrast agent solutions at concentrations of 45-50% contrast agent, regardless of the presence or absence of Tween™ 20 surfactant. The conditions for saturation appeared to be dependent upon the initial pH of the particles, the pH used during the procedure and the corresponding saturation with methacrylic acid groups in the particle. At pH below 3.6, constant exchange between protons and cations was observed. As a result, more beneficial results were shown at pH above about 3.6 and below about 6.6 to temper the amount of cesium. Within the preferred range, buoyancy can be varied. At reasonably neutral levels, based on test at pH of 7.4, the microparticles did not lose their buoyancy after storage in the contrast agent buffered solution over night.

EXAMPLE 14

[00122] Further compressibility and mechanical property testing were done on microspheres in accordance of Samples B and/or C of Example 12. A pressure test stand which was used for further evaluation is shown in Fig. 8. An automated syringe plunger 2 having a motor 4 for providing a variable feed rate of 0 to 250 mm/h and a gear box 6 was further equipped with a Lorenz pressure transducer 8 capable of measuring forces in the 0 to 500 N range. The syringe plunger 2 was in communication with a syringe body 10 as shown. The digital output of the transducer was recorded using a personal computer. The syringe body 10 was filled with 5 ml of a solution of contrast agent in isotonic phosphate buffer/surfactant (Tween™ 20) solution in a concentration of about 30-32 volume percent contrast agent. Microparticles were provided to the syringe as well in an amount of 56 mg dry mass. The syringe contents were then injected through the microcatheter 12 which was attached to the distal end 14 of the syringe. The microcatheter had a lumen diameter of 533 μm. The force needed to push the microparticles through the catheter into the Petri

dish 16 (shown for receiving microparticle solution) was measured and recorded as pressure.

[00123] In order to make certain calculations, the following information was applied as based on typical use of microspheres for embolization. Typically such microspheres have a water content of about 90% such that a vial for embolization would therefore contain 0.2 mg of embolization particles in 9.8 ml of injection liquid (2 ml of hydrated microparticles in 8 ml supernatant liquid). Standard preparation procedures include adding 8 ml of Imeron® 300 contrast agent to the contents of a single vial. This would provide an equilibrium concentration of contrast agent of 8 ml/(9.8 ml + 8 ml) = 44.9 volume percent within an injection solution. The solution is typically drawn up in 1 ml syringes for final delivery. The injection density thus equals:

$\rho = V_{Emb}/V_{Tot} = 2$ ml/18ml = 0.111 Embolization agent per volume fraction.

[00124] The Sample C spheres demonstrated approximately the same equilibrium water content as typical embolization spheres. To achieve the same injection density desired for typical surgical procedures, 56 mg of Sample C microspheres were added to 5 ml of a 31 volume percent contrast agent solution in isotonic phosphate buffer and surfactant as noted above.

[00125] The Sample B and C microspheres were evaluated in different microcatheters of equal lumen diameter at a pH of 7.4. Injections in both the horizontal and vertical direction were made under different buoyancy levels and using different swelling levels (based on pH of 6.0 in contrast to pH 7.4). The results demonstrated that as long as the diameter of the microspheres was below the internal diameter of the microcatheter, the microparticles passed through the catheter without additional frictional force in the same manner as the reference solution. An increase to about 1.0 to 1.4 kg gravitation force was measured when the microparticle diameter reached the same dimension as the lumen diameter. At roughly 20% compression, forces of about 1.5-2.3 kg were needed to overcome frictional forces within the catheter. Forces greater than 5 kg were taken as a guideline for moderate to high injection pressures. When particles are heavier than the injection medium, clogging was observed when injecting in the vertical position. When injecting the microparticles in the horizontal position, it was observed that serious clogging was alleviated and that larger volumes were injectible over time.

[00126] Injection pressure was further minimized when a lower pH (reduced swelling) was used in combination with horizontal injection such that the injection pressures were comparable to the injection media itself. In addition, injection of Sample C microparticles also exhibited a good injection pressure pattern at a physiological pH. The catheter entrance did not clog and each peak in the curve corresponded to either a single microparticle or number of particles passing through the catheter.

[00127] The results of the various catheter simulation tests shows that the invention can be used to form injectible microparticles having a density which substantially matches the density of the injection medium for embolization use. The particles' compressibility can further be such that it can be injected without forces over more than about 5 kg on the syringe plunger. The pH of the injection medium can be taken down to about 6 or injections can be done horizontally to increase the ease of passage of Sample B and C microparticles through the catheter. Once within the blood stream, the particles can expand to their original size in the pH 7.4 environment.

[00128] Additional swelling tests were conducted on the microparticles of Sample C and it was observed that when ion concentrations were low, swelling increased. In higher concentrated solutions, swelling decreased. Continued dilution of the microparticles of Sample C in a buffer solution led to an increase from 17% to 20% in size of the microparticles. When mixed into an isotonic phosphate buffer solution, the microparticles initially increase in size between 83.8 and 97%, wherein in deionized water, size increases are from about 116.2 to about 136.6%, referring to the dry particles.

[00129] In further testing to evaluate the compressibility of the microparticles of Sample C, the syringe pressure test stand of Fig. 8 was used, however, an optical microscope was used to evaluate the microparticles as they passed through a progressively narrowed pipette which was attached to polyethylene tubing connected to the syringe containing a phosphate buffer solution suspension of microparticles of Sample C. The pipette narrowed to an inner diameter of 490 μm and the pipette was mounted to a Petri dish such that the narrowest part was submerged in phosphate buffer solution to avoid optical distortion and to collect the liquid ejected from the pipette during measurement. Optical microscope pictures were taken of the microparticles passing through the pipette before and during compression. In observing the microparticles, none of them underwent a fracture, nor did

they form debris or coating delamination after passing through the narrow site. Microparticles which were chosen to be deliberately too big for the narrow site (for a compression of about 40%) did not break or rupture, but clogged the narrow site instead. The maximum compressibility under a reasonable amount of force on the microparticles while still allowing the microparticles to pass through the catheter was about 38.7%. Based on these evaluations, the microparticles according to Sample C demonstrate properties that would allow particles which are too large to clog the catheter rather than break up and cause potential damage to the patient. The test results provided suggested preferred use parameters for Sample C microparticles for embolization use as shown in Table 2 below:

TABLE 2

Particle Radius (μm)	Constriction (μm)	Compression (%)	Force Needed (kg)
340	540	25.9 and 26.5	2.58 and 1.92
360	540	33.3	3.19
330	540	22.2	2.83
330	540	22.2	2.14
370	540	37.0 and 37.3	3.59 and 2.77
330	540	22.2	2.08
320	540	18.5 and 18.4	1.61 and 1.38
330	540	22.2	1.71

[00130] Sample C microparticles were further subjected to mechanical and thermal stress stability testing. Microparticles, after passing through a Terumo Progreat Tracker catheter were washed with deionized water to remove residual buffer solution along with contrast agent. They were dehydrated for 12 h at 60° C and then transferred to an SEM for surface analysis. They were compared with particles from the original batch of microparticles which had undergone the same hydration/dehydration cycle in milliQ ultrapure water, but which had not been passed through the catheter. Figs 9A and 9B show the surface of the Sample C microparticles just after the hydration/dehydration cycle and the film thickness of an exemplary Sample C microparticle, respectively. SEMs after passing through a catheter at various magnifications (Figs. 10A, 10B, 10C and 10D) show that the coating did not delaminate (Fig. 10A). Some microparticles did demonstrate some stretching out in the coating film (Figs. 10B and 10C). However, a closer magnification as in Fig. 10D demonstrates that the morphology of the coating layer is still intact.

[00131] A sterilizer was filled with 2 l of deionized water and 10 vials each having 56 mg of Sample C microparticles in 3.3g of solution of isotonic phosphate buffer/surfactant (Tween™ 20) and turned on. The water boiling point was reached about 15 min. after the start of the sterilizer, and temperature was held at that point for 3 min. to remove air by water vapor. The vessel was then sealed shut to raise pressure and temperature to 125° C and 1.2 bar pressure. This took approximately 10 min. The temperature was then maintained for 15 min, and then the vessel was shut down for a cooling phase. A temperature of 60° C was reached about 30 min later, after which the vessel was vented, the samples withdrawn and the vessel shut tightly. A sample vial was opened, and the supernatant liquid decanted. The microparticles were washed with deionized water. After dehydration, they were subjected to measurement using an SEM. The results demonstrated only a small number of delaminated coatings on the microparticles under such thermal stress (see Fig. 11A in the strong white contrast portion). The overall percentage of such microparticles was only about 5 to 10%. Close up, the film delamination which did occur appears to have occurred along crystalline-amorphous domain boundaries in the poly[bis(trifluoroethoxy)phosphazene] coating (see Fig. 11B). Most of the microparticles showed only minor defects (such as a minor circular patch being missing), but no damage to the hull of the microparticles (see Figs. 11C and 11D).

EXAMPLE 15

[00132] Microparticles were formed in accordance with a preferred embodiment herein. A deionized water solution of polyvinyl alcohol (PVA) was prepared using about 23g of PVA of weight average molecular weight of about 85,000-124,000, which PVA was about 87-89% hydrolyzed and 1000 g water. A phosphate buffer solution was prepared using 900 g deionized water, 4.53 g disodium hydrogen phosphate, 0.26 g sodium dihydrogen phosphate and 0.056 g ethylenediamine tetraacetic acid (EDTA). Methyl methacrylate (MMA) monomer was vacuum distilled prior to use.

[00133] Polymerization was carried out in a three-necked, round-bottomed, 2000-ml flask with a KPG mechanical stirring apparatus attached. The flask was also equipped with a thermometer, reflux condenser and a pressure release valve with a nitrogen inlet. The polymerization process further utilized 100 ml of the PVA solution prepared above,

900 ml of the phosphate buffer solution, 0.65 g of dilauroyl peroxide, 200.2 g methacrylic acid methyl ester and 2.86 g triethylene glycol dimethacrylate.

[00134] The PVA and buffer solutions were provided to the reactor flask. The distilled MMA and triethylene glycol dimethacrylate were introduced, dilauroyl peroxide then added to the same flask and the components were agitated to ensure dissolved solids. The reaction flask was flushed with argon and the stirrer speed set to at 150 rpm to produce particle sizes of a majority in the range of 300-355 μm. Stirring continued for approximate 5 minutes. The stirrer was then set to 100 rpm and argon flushing was discontinued. The reaction flask was then subjected to a water bath which was heated to 70° C and held at approximately that temperature for about 2 hours. The temperature of the bath was then increased to 73° C and held for an hour, then the water bath temperature was raised again to 85° C and held for another hour. The stirring and heat were discontinued. The solution was filtered and the resulting polymethylacrylate microparticles were dried in an oven at 70° C for about 12 hours. The microparticles were subjected to sieving and collected in size fractions of from 100-150; 150-200; 200-250; 250-300; 300-355; 355-400; and 400-450 μm with a maximum yield at 300-355 μm.

[00135] The PMMA microparticles thus formed were then hydrolyzed. A portion of 100 g 250-300 μm sized microparticles, 150 g potassium hydroxide and 1400 g of ethylene glycol were added to a 2000 ml flask, reflux condenser with drying tube connected, and the mixture was heated at 165° C for 8 hours for full hydrolysis. The mixture was allowed to cool to room temperature, solution decanted and the microparticles were washed with deionized water. The procedure was repeated for other calibrated sizes of microparticles (the following reaction times applied: 300-355 micron particles: 10 hours; 355-400 micron particles: 12 hours and 400-455 micron particles: 14 hours).

[00136] The microparticles were finally acidified with hydrochloric acid to a pH of 7.4, and dried in an oven at approximately 70° C.

EXAMPLE 16

[00137] Microparticles formed in accordance with Example 15 were then esterified in this Example. For esterification surface treatment, 800 g of dried microparticles from Example 15 were weighed in a 2L reaction vessel with a reflux condenser. 250 g thionyl

chloride in 1.5 L diethyl ether were added under stirring. Stirring was continued at room temperature for 20 hours. The solvent and volatile reactants were removed by filtration and subsequent vacuum drying. Then 500 g trifluoroethanol in 1.5 L ether were introduced and the suspension stirred for another 20 hours at room temperature. The particles were finally dried under vacuum.

EXAMPLE 17

[00138] In an alternative surface treatment to Example 16, 800 g dried microparticles from Example 15 were reacted with 1140 g trifluoroethanol and 44 g sulfuric acid added as a catalyst. The mixture was stirred for 20 hours at room temperature, filtered and dried under vacuum.

EXAMPLE 18

[00139] 800 g of dry PMMA potassium salt microparticles which were partially esterified with trifluoroethanol as described above in Examples 15-16 were spray coated with poly[bis(trifluoroethoxy)phosphazene] in an MP-1 Precision Coater™ fluidized bed coating apparatus (available from Aeromatic-Fielder AG, Bubendor, Switzerland). The particles were picked up by an air stream (40-60 m^3/h, 55° C incoming temperature) and spray coated with poly[bis(trifluoroethoxy)phosphazene] solution microdroplets from an air-fluid coaxial nozzle. The solution composition was 0.835 g poly[bis(trifluoroethoxy) phosphazene], 550 g ethyl acetate and 450 g isopentyl acetate. It was fed through the nozzle's 1.3 mm wide inner bore at a rate of 10-30 g/min. At the nozzle head, it was atomized with pressurized air (2.5 bar). The total amount of spray solution (3kg) was calculated to coat the particle with a 150 nm thick poly[bis(trifluoroethoxy)phosphazene] film.

EXAMPLE 19

[00140] The dry potassium salt microparticles of Examples 15-16, which were partially esterified with trifluoroethanol as described above, were spray-coated with diluted poly[bis(trifluoroethoxy)phosphazene] solution in ethyl acetate in a commercially available fluidized bed coating device (see Example 16). 100 mg of such coated, dried microparticles as well as 100 mg of uncoated, dried PMA potassium salt microparticles which were partially esterified with trifluoroethanol, were immersed in about 30% aqueous

cesium chloride solution, prepared by dissolving 30.0 g cesium chloride in 100 ml deionized water. The supernatant liquid was decanted after 10 min. equilibrium time and the microparticles were washed thoroughly with deionized water, equilibrated for another 10 min., decanted and suspended in 3 ml surfactant free phosphate buffer solution at a pH of 7.4. Density of the particles in solution was measured for matching density in a contrast agent solution. To each type of microparticle was added a contrast agent solution which included a ratio of 3.5 ml of Imeron® 300 contrast agent (density 1.335 g/ml) and 4 ml phosphate buffered saline (density 1.009 g/ml). Both hydrogel types reached buoyancy at levels of 45-50% contrast agent in solution. This corresponds to an increased density of the microparticles of 1.16 g/ml.

EXAMPLE 20

[00141] Microparticles were formed in accordance with the procedure of Example 15 with the exception that an exterior barium sulfate coating was prepared on the microparticles after neutralization of the particles and the microparticles were not dried after neutralization prior to the barium sulfate coating step. To prepare the barium sulfate coating, 2500 ml hydrated particles were subjected to 2000 ml of 0.5 M sodium sulfate (Na_2SO_4) solution and saturated for 4-12 hours. To the particle suspension was then slowly added 1950 ml of 0.5 M barium chloride ($BaCl_2$) solution under stirring at room temperature. After washing with excess deionized water, the resulting particles in a swollen state included a barium sulfate powder coated surface. The particles were then dried and esterified in the manner noted above in Example 16. The particles were then coated using the fluidized bed process of Example 21 below. The resulting microparticles were externally coated with a non-adhesive barium sulfate powder. Barium sulfate coatings prepared in accordance with this invention and procedure are capable of preventing particle agglomeration during drying and also increase density. The concentration and ratios of barium sulfate may be varied to provide different results and a use of an excess of sodium sulfate can minimize residual barium chloride. The particles formed in accordance with this example were effectively washed with hot water to minimize excess barium sulfate powder that may contaminate vials, etc. The barium sulfate works effectively to prevent adhesion of particles prior to drying to assist in fluidization of the hydrated microparticles.

EXAMPLE 21

[00142] Fluidized bed coating of barium sulfate powdered beads was performed using polymethacrylate beads with a surface layer of barium sulfate formed in accordance with Example 20 but an excess of barium chloride was used such that barium ions diffused inside the core and formed a precipitate inside the hydrogel core.

[00143] In preparing the particles, the same procedure for barium sulfate coated particles set forth in Example 20 was repeated with the exception that the order of addition was reversed. Thus, 2500 ml hydrated microparticles were suspended in 2500 ml deionized water and slowly, 5 mol % (200 ml) of a 0.5 M ($BaCl_2$) were added slowly under stirring. The addition was performed within a time period of three minutes to prevent irreversible barium acrylate formation taking place. The suspension was then immediately quenched with the double amount (400 ml) of 0.5 M sodium sulfate (Na_2SO_4) solution under stirring at room temperature. Afterwards, the particles were washed three times with 2 L of deionized water each. This procedure precipitated barium sulfate inside the particles.

[00144] The resulting precipitate was precipitated within the pores of the hydrogel core and could not be removed by multiple washings with water. The particles thus formed were found to have a permanent increased density in contrast to unmodified particles. The density increase was controllable by the molar amount of barium chloride used. Amounts ranging from 0-15 mol % of barium chloride were used reproducibly with this procedure. It was observed during evaluations of this procedure that, if the time period of addition exceeded 5 minutes, based upon the diffusion speed of barium chloride within the particles, the outer pores of the hydrogel core became irreversibly crosslinked, thereby preventing the barium sulfate precipitate inside from leaching out. This effect was visible by optical microscopy as the "diffusion front" of the barium sulfate was clearly visible as a white band inside the particle, whereas the surface remained clear.

[00145] Both Examples 20 and 21 provided particles having anti-adhesive properties that tend not to agglomerate during drying processes; therefore avoiding surface damage. Generally, such an advantage helps minimize the amount of particles needed for a fluidized bed procedure as the particles can be fluidized without being completely dried. The residual water content may be increased up to 1:1 based on dry weight without

agglomeration. The Examples also produce particles with increased density properties wherein the density change appears to be permanent.

[00146] It should also be understood according to this disclosure that generally when applying the procedures noted herein, barium sulfate may be introduced in accordance with the invention in a range of from 0 to about 100 mol%, and preferably 0 to about 15 mol% to provide particles that have preferred elasticity, density and mechanical stability properties.

[00147] The particles formed according to this Example having a barium sulfate load inside the core were then esterified according to Example 16 and vacuum-dried. 300g of the dry beads were suspended in 300 g water which was completely absorbed by the polymethacrylate cores within less than 1 min while the barium sulfate powdered particle surface appeared dry and the particles showed no tendency to agglomerate.

[00148] The particles (now 600 g) with 50 weight percent (wt%) water inside were spray coated with APTMS/ poly[bis(trifluoroethoxy)phosphazene] in an MP-1 Precision Coater™ fluidized bed coating apparatus according to Example 18 with the exception that an additional aminosilane adhesion promoter was used. The process equipment used was the same as that of Example 18, but the coating provided included three different layers. A bottom coating of 3-aminopropyltrimethoxysilane (APTMS) adhesion promoter was provided upon which was a second coating layer of a mixture of APTMS and poly[bis(trifluoroethoxy)phosphazene] and a third, top coating layer of poly[bis(trifluoroethoxy)phosphazene]. All three spray solutions were prepared by dissolving the coating material in isopentyl acetate and ethyl acetate in a 1:1 weight percentage ratio mixture. The first solution included 35 µl APTMS dissolved in 200 g acetate mixture. The second solution included 25 µl APTMS and 125 mg poly [bis (trifluoroethoxy)phosphazene] in 150 mg of the acetate mixture and the third included 50 mg poly [bis (trifluoroethoxy)phosphazene] in 60 g of the acetate mixture. The spray solution quantities and concentrations refer to the coating of a 300 g batch with 350 µm particles. The absorbed water evaporated at a rate of 5 - 10 g/min. The process was stopped after 30 min when the coating thickness reached 100 nm and the residual water content was 18.4 wt%.

EXAMPLE 22

[00149] The absorption of organic dyes was tested on microparticles formed according to Example 15. To 2 ml of phosphate buffered saline solution containing 1 ml of hydrated beads was provided an amount of 5-10 µl of the respective dye as a 10 millimolar solution in ethanol. The samples were incubated for 30-60 minutes at room temperature under gentle shaking of the vial. Supernatant liquid was discarded and particles were washed three times with 2 ml of deionized water, saline or PBS buffer solution prior to visualization with optical and fluorescence microscopy. The dyes tested included triphenylmethane derived dyes such as Fluoescein diacetate and Rhodamin 6G which were evaluated along with carbocyanine based dyes such as DiI. The triphenylmethane based Fluorecein and Rhoamine dyes exhibited a specific affinity for the hydrophilic PMMA hydrogel core through ionic interactions. They were able to easily withstand the rigorous conditions of repeated washing and steam sterilization without substantial leaching.

The carbocyanine dye DiI on the other hand exhibited a high selectivity for the hydrophobic poly[bis(trifluoroethoxy)phosphazene] shell, without penetrating the hydrophilic PMAA core material. Thus with the subsequent staining employing the combination of DiI and Fluorescein diacetate both core and shell could be simultaneously visualized employing a fluorescence optical microscope. As a result, this procedure provides a fast, sensitive fluorescence-staining assay for the PMAA particles that makes core and shell simultaneously visible under conditions encountered in actual application. This procedure further enables assessment of the mechanical-elastic stress or damage to the poly[bis(trifluoroethoxy)phosphazene] shell. It further shows the affinity of certain classes of dyes for the various components of the particle.

[00150] Use of these and other dyes may be used to visually identify selected microspheres, which may be provided and dyed for identification to indicate certain sizes of microspheres for use in selected applications. Color-coding may also be used to identify selected microspheres on the basis of other properties, such as permanent or absorbable microsphere structures.

[00151] In various embodiments according to the present invention, microspheres may be produced in calibrated sizes ranging from about 1 to about 10,000 nanometers in diameter. In one embodiment of the present invention, microspheres of the present

invention may be provided in sizes of about 40, about 100, about 250, about 400, about 500, about 700, and about 900 nanometers in diameter, with a visually distinctive color imparted to each size of microsphere. Other sizes, size ranges, and calibrated sized microspheres lacking color dye are also included in the present invention. Microspheres of the present invention may also be provided in customized sizes and/or with customized colors as specified by a user for specific clinical diagnostic or therapeutic applications.

EXAMPLE 23

[00152] In certain exemplary embodiments of the present invention, microspheres containing dyes of one or more colors may be delivered into the dermis of a biological host's skin in a desired pattern to create a tattoo or other marking that is visible to an observer. Such delivery may be effected using needle injection, jet injection, implantation, adhesion, ingestion, or any other delivery method or technique.

[00153] As shown in Fig. 12, a cross section of skin of a mammalian biological host comprises a skin surface **105**, an epidermis **110**, a dermis **115**, and subcutaneous tissue **120**. An injection or delivery needle **130** may be inserted into the dermis **115** and microspheres **135** containing dyes may be deposited therein in a desired pattern to create a tattoo or other marking as desired.

EXAMPLE 24

[00154] In some preferred embodiments of the present invention, microspheres or other structures are provided, comprising an outer shell or coating of poly[bis(trifluoroethoxy)phosphazene] and a hydrogel core further comprising a chromophoric agent or other dye permanently covalently bound to the hydrogel core material.

[00155] Chromophoric agents according to the present invention, and particularly organic molecules with chromophore groups attached, will absorb electromagnetic light depending on what type of chromophores are attached thereto. The absorbed energy may be dissipated by different modes including, but not limited to, molecular bond vibration, rotation, electronic excitation, and fluorescence.

[00156] When the absorbed energy exceeds bond energies within the chromophore molecule, the molecule can break up. If the molecule is electronically excited, it may form highly unstable transition states which do not necessarily recombine to the state the molecule was in before. This process may lead to a gradual degradation of the chromophoric agent under light irradiation, resulting in eventual loss of color intensity [i.e., photobleaching]. Photobleaching is typically observed with molecules that show fluorescence emission when excited at higher energy wavelengths. Photobleaching may be triggered by high intensity laser light, UV radiation, or exposure to other forms of electromagnetic radiation

EXAMPLE. 25

[00157] In other embodiments of the present invention, devices comprising radio frequency identification electronic chips or nanochips or external casings containing such electronic chips or nanochips may be provided with an external coating of poly[bis(trifluoroethoxy)phosphazene] or derivatives thereof, and applied within a biological host body at any desired location. The application of such devices to the biological host body may be accomplished by implantation, injection, ingestion, adhesion, or any other applicable technique to deliver the devices to their desired locations. In use, external radio frequency transmitters or receivers may be used to send signals to, or receive signals from such electronic chips or nanochips, allowing an observer to identify an individual biological host, or to track or monitor location or other factors related to the biological host.

[00158] As shown in Fig. 13, a cross section of skin of a mammalian biological host comprises a skin surface **105**, an epidermis **110**, a dermis **115**, and subcutaneous tissue **120**. Within the dermis **115**, a deposited radio frequency identification electronic chip **145** is shown, encapsulated by a polymer shell **140** of the present invention.

EXAMPLE 26

[00159] In yet other embodiments of the present invention, microspheres or other structures containing dyes according to the present invention may be placed in tissue or within skin to serve as port markers for radiation therapy, or to otherwise mark a specific anatomic area.

EXAMPLE 27

[00160] In certain exemplary embodiments of the present invention, microspheres containing dyes of one or more colors may be delivered into the dermis of a biological host's skin in a desired pattern to create a tattoo or other marking that is visible to an observer. Such delivery may be effected using needle injection, jet injection, implantation, adhesion, ingestion, or any other delivery method or technique.

EXAMPLE 28

[00161] In yet other exemplary embodiments of the present invention, a hand-held spectrophotometer that records data from a desired area of a biological host's skin is used in conjunction with a computerized color formulation system. Based on this color measurement, a color formula will be calculated by the computer, and appropriate dyes will be mixed to produce pigmented microspheres to match the color of the target skin.

[00162] Alternately, microspheres of the present invention may be provided in selected colors approximating common skin tones.

[00163] Using such skin color-matched or approximately colored microspheres, an existing tattoo or other dermal discoloration may be treated by deposition of such microspheres into the affected dermis above the prior marking to mask the tattoo or discolored area.

[00164] Referring now to Fig. 14, a cross section of skin of a mammalian biological host comprises a skin surface **105**, an epidermis **110**, a dermis **115**, and subcutaneous tissue **120**. As shown in Fig. 14. the dermis **115** contains pigment **125** representing a prior tattoo. An injection or delivery needle **130** may be inserted into the dermis **115** and microspheres **135** containing dyes may be deposited therein in a desired pattern to cover the pigment **125**.

EXAMPLE 29

[00165] As provided herein, microspheres of the present invention may further be provided with fluorescent or phosphorescent dyes to allow selective identification of dermal markings made using such microspheres upon exposure to ultraviolet radiation.

Such markings would normally be undetectable to visual inspection, but would exhibit light emission in the visible spectrum when illuminated by UV light.

EXAMPLE 30

[00166] Fig. 15 shows a schematic view of an exemplary automated inoculation system utilizing the identifying microparticles of the present invention. In Fig. 15, an automated inoculation system **1000** comprises an inoculation gun **1005** which further comprises a shaft **1010**, a handle **1015**, an activation trigger **1035**, and a patient interface **1020**. The patient interface **1020** comprises one or more sensors **1025** and one or more injection ports **1030**. The one or more sensors **1025** are in electronic communication with a central processing unit **1040** comprising a software-driven electronic computer system. The central processing unit **1040** is further in electronic communication with a mixing system **1045** which draws medications, vaccines, or other therapeutic agents from one or more reservoirs **1050**. In some embodiments of an automated inoculation system according to the present invention, a scale **1055** may also be electronically connected to and integrated with the central processing unit **1040**.

[00167] In use, an operator holds the inoculation gun **1005** against the skin of an animal or human patient and activate the activation trigger **1035**. The sensor **1025** senses and electronically reads one or more electronic messages contained within identifying microparticles of the present invention. Such identifying microparticles may be one or more devices comprising radio frequency identification electronic chips or nanochips or external casings containing such electronic chips or nanochips with an external coating of poly[bis(trifluoroethoxy)phosphazene] or derivatives thereof, and previously applied within the patient's body at a desired location. The sensor **1025** transmits this data to the central processing unit **1040** where the computer identifies the patient in a stored database and calculates the types and doses of therapeutic agents needed by that individual patient. In various embodiments, the central processing unit **1040** may also receive data from a scale **1055** to aid in calculating the appropriate doses for that individual in real time. The central processing unit **1040** then exchanges electronic signals to a computer-controlled mixing system **1045** which which draws medications, vaccines, or other therapeutic agents from one or more reservoirs **1050** in an appropriate dose for the individual patient, and

conveys the therapeutic agents to the inoculation gun **1005** where the inoculation is delivered through the one or more injection ports **1030**.

[00168] The exemplary automated inoculation system of Fig. 15 and other embodiments of the present invention allow a single operator to quickly and accurately deliver appropriately individualized inoculations in precise doses and combinations to a large number of individual patients.

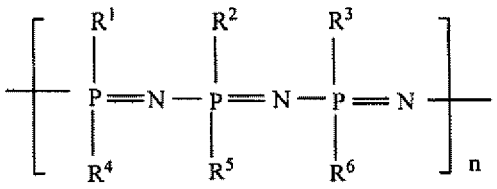
[00169] It will be appreciated by those possessing ordinary skill in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the appended claims.

CLAIMS

We Claim:

1. Polymeric particles comprising a core, a polyphosphazene coating, and one or more marking or masking agents, wherein:

the polyphosphazene of the polyphosphazene coating has the formula:



(I)

n is 2 to ∞; and

R^1 to R^6 are each selected independently from alkyl, aminoalkyl, haloalkyl, thioalkyl, thioaryl, alkoxy, haloalkoxy, aryloxy, haloaryloxy, alkylthiolate, arylthiolate, alkylsulphonyl, alkylamino, dialkylamino, heterocycloalkyl comprising one or more heteroatoms selected from nitrogen, oxygen, sulfur, phosphorus, or a combination thereof, or heteroaryl comprising one or more heteroatoms selected from nitrogen, oxygen, sulfur, phosphorus, or a combination thereof.

2. The particles of claim 1, wherein at least one of R^1 to R^6 is an alkoxy group substituted with at least one fluorine atom.

3. The particles of claim 1, wherein the polyphosphazene is poly[bis(2,2,2-trifluoroethoxy)phosphazene] or a derivative of poly[bis(2,2,2-trifluoroethoxy)-phosphazene], and wherein the polyphosphazene is provided as a coating substantially enclosing the core.

4. The particles of claim 1, wherein the core is a hydrogel and wherein the one or more marking or masking agents comprises one or more chromophoric agents.

5. The particles of claim 4, wherein the one or more chromophoric agents are covalently bound to the hydrogel core material.

6. The particles of claim 4, wherein the one or more chromophoric agents are selected from fluorescent dyes, phosphorescent dyes, or a combination thereof.

7. The particles of claim 4, wherein the one or more chromophoric agents may be permanent chromophoric agents or capable of degradation *in vivo*.

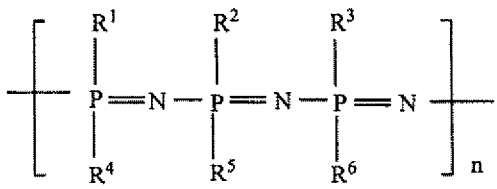
8. Polymeric particles comprising poly[bis(trifluoroethoxy)phosphazene] and one or more electronic chips or nanochips containing identifying electronic information.

9. The particles of claim 8, wherein the one or more electronic chips or nanochips emit a radiofrequency signal containing the identifying electronic information.

10. A method of marking an individual patient, the method comprising:

a. providing polymeric particles comprising a core, a polyphosphazene coating, and one or more marking agents, wherein:

the polyphosphazene of the polyphosphazene coating has the formula:



(I)

,

n is 2 to ∞; and

R^1 to R^6 are each selected independently from alkyl, aminoalkyl, haloalkyl, thioalkyl, thioaryl, alkoxy, haloalkoxy, aryloxy, haloaryloxy, alkylthiolate, arylthiolate, alkylsulphonyl, alkylamino, dialkylamino, heterocycloalkyl comprising one or more heteroatoms selected from nitrogen, oxygen, sulfur, phosphorus, or a combination thereof, or heteroaryl comprising one or more heteroatoms selected from nitrogen, oxygen, sulfur, phosphorus, or a combination thereof;

b. delivering the polymeric particles into the dermis of a biological host's skin in a desired pattern; and

c. creating a tattoo or other marking that is visible to an observer.

11. The method of claim 10, wherein the core is a hydrogel and wherein the one or more marking or masking agents comprises one or more chromophoric agents.

12. The method of claim 11, wherein the one or more chromophoric agents are covalently bound to the hydrogel core material.

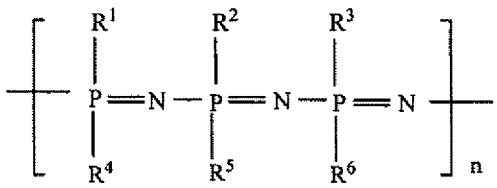
13. The method of claim 11, wherein the one or more chromophoric agents are fluorescent or phosphorescent dyes.

14. The method of claim 11, wherein the one or more chromophoric agents may be permanent or capable of degradation *in vivo*.

15. A method of masking an individual patient, the method comprising:

a. providing polymeric particles comprising a core, a polyphosphazene coating, and one or more marking or masking agents, wherein:

the polyphosphazene of the polyphosphazene coating has the formula:



(I)

,

n is 2 to ∞; and

R^1 to R^6 are each selected independently from alkyl, aminoalkyl, haloalkyl, thioalkyl, thioaryl, alkoxy, haloalkoxy, aryloxy, haloaryloxy, alkylthiolate, arylthiolate, alkylsulphonyl, alkylamino, dialkylamino, heterocycloalkyl comprising one or more heteroatoms selected from nitrogen, oxygen, sulfur, phosphorus, or a combination thereof, or heteroaryl comprising one or more heteroatoms selected from nitrogen, oxygen, sulfur, phosphorus, or a combination thereof;

b. delivering the polymeric particles comprising poly[bis(trifluoroethoxy)phosphazene], a core, and one or more marking agents into the dermis of a biological host's skin in a desired pattern; and

c. obscuring a tattoo or other marking with the pattern such that it is rendered less visible to an observer.

16. The method of claim 15, wherein the polyphosphazene is poly[bis(2,2,2-trifluoroethoxy)phosphazene] or a derivative of poly[bis(2,2,2-trifluoroethoxy)-phosphazene], and wherein the polyphosphazene is provided as a coating substantially enclosing the core.

17. The method of claim 15, wherein the core is a hydrogel and wherein the one or more marking agent comprises one or more chromophoric agents.

18. The method of claim 17, wherein the one or more chromophoric agents are covalently bound to the hydrogel core material.

19. The method of claim 17, wherein the one or more chromophoric agents may be permanent or capable of degradation *in vivo*.

20. An automated identification and inoculation system , comprising:

a. a sensor capable of sensing identifying electronic information within one or more electronic chips or nanochips contained within polymeric particles implanted in an individual patient, wherein the polymeric particles comprise poly[bis(trifluoroethoxy)phosphazene];

b. an injector unit capable of administering a dose of one or more therapeutic or prophylactic agents to the patient;

c. a mixer capable of mixing the dose from one or more therapeutic or prophylactic agents and delivering the dose to the injector unit;

d. a central processing computer capable of:

i. receiving the identifying electronic information from the sensor,

ii. comparing the identifying electronic information with stored data in a database,

iii. determining the desired dose of the one or more therapeutic or prophylactic agents individually appropriate for the individual patient,

iv. transmitting instructions to the mixer to prepare the desired dose of the one or more therapeutic or prophylactic agents and deliver the dose to the injector unit, and

v. transmitting instructions to the injector unit to inject the dose into the individual patient.

21. The automated identification and inoculation system of claim 20, further comprising a scales to provide real-time data of the weight of the individual patient to the central processing computer to allow a weight-based calculation of the desired dose of the one or more therapeutic or prophylactic agents individually appropriate for the individual patient,

22. The automated identification and inoculation system of claim 20, further comprising:

a. a handheld inoculation gun comprising the sensor, the injector unit with one or more injection ports, an activation trigger, and electronic connections among the sensor, the central processing computer, the activation trigger, and the mixer unit; and

b. a fluid conduit connecting the mixer unit and the injector unit.

23. The automated identification and inoculation system of claim 20, wherein the sensor is capable of reading a tattoo or other skin surface identifying mark on the individual patient.

24. The automated identification and inoculation system of claim 23, wherein the tattoo or other skin surface identifying mark on the individual patient is comprised of intradermally injected particles comprising a core, a poly[bis(trifluoroethoxy)phosphazene] coating, and one or more marking agents into the dermis of the individual patient's skin in a desired pattern.

25. The automated identification and inoculation system of claim 24, wherein the one or more marking agent comprises one or more chromophoric agents.

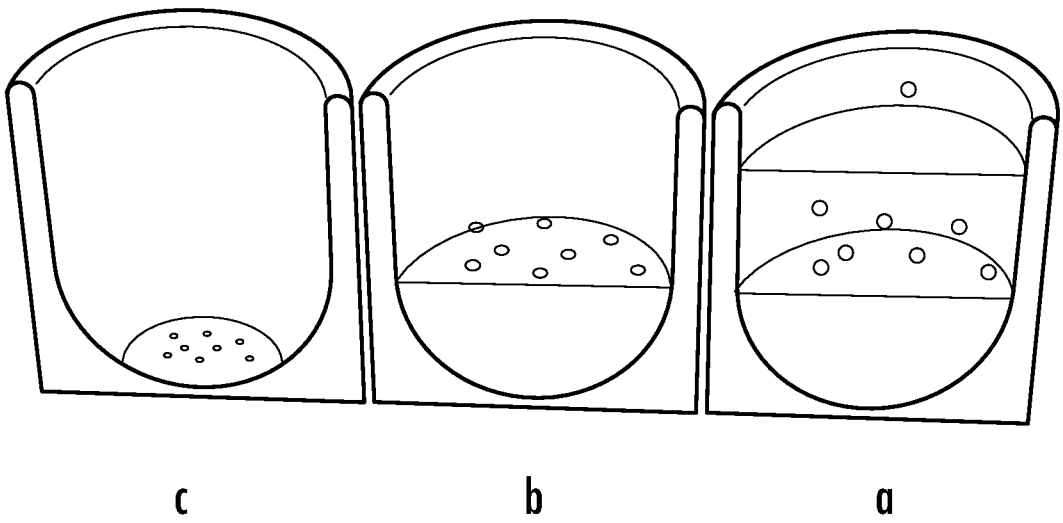


Fig. 1

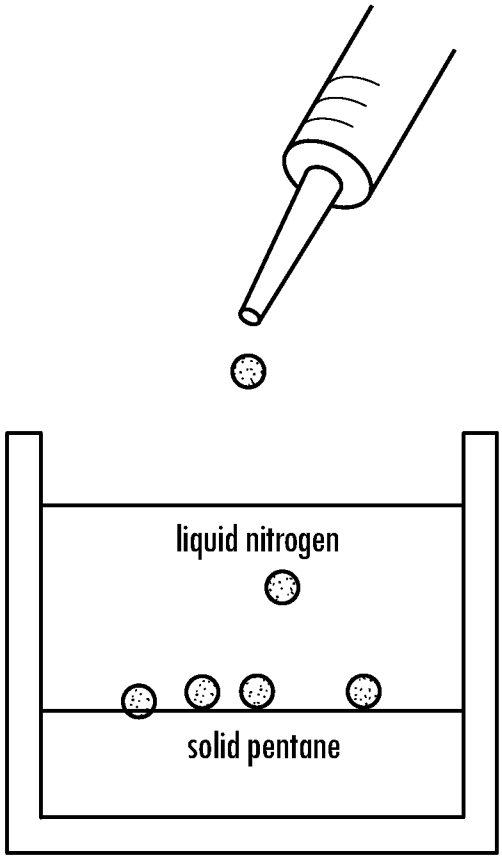


Fig. 2

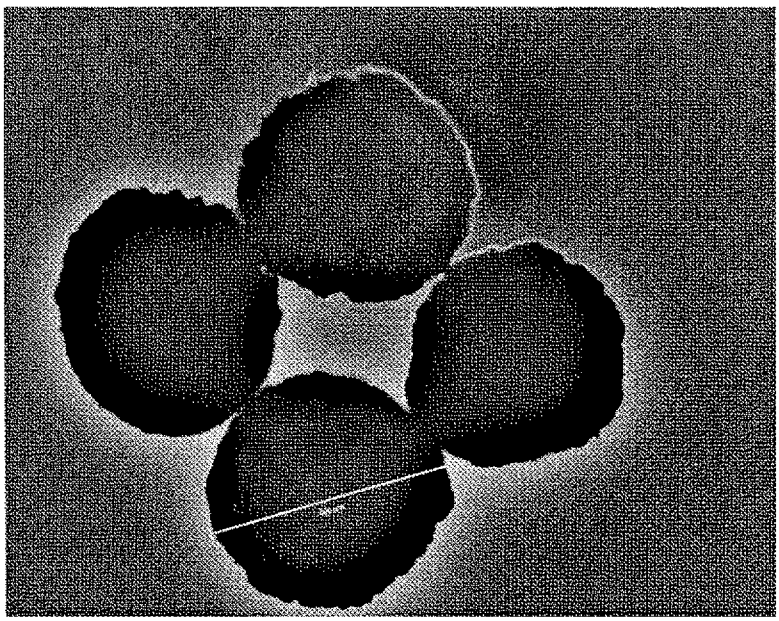


Fig. 3A

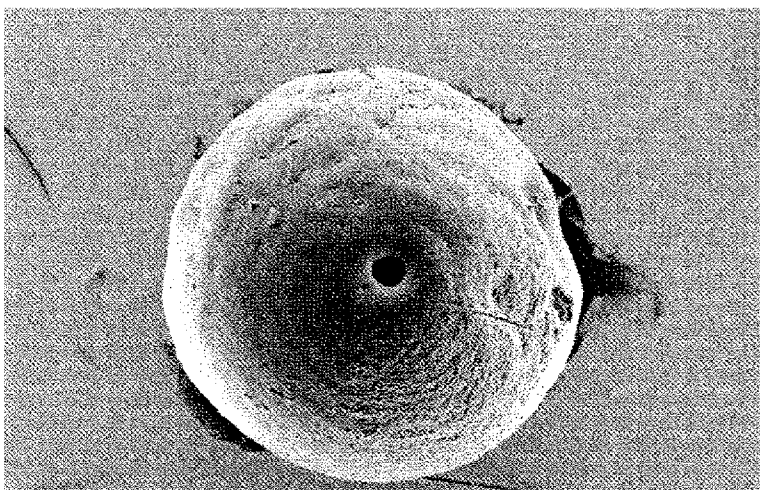
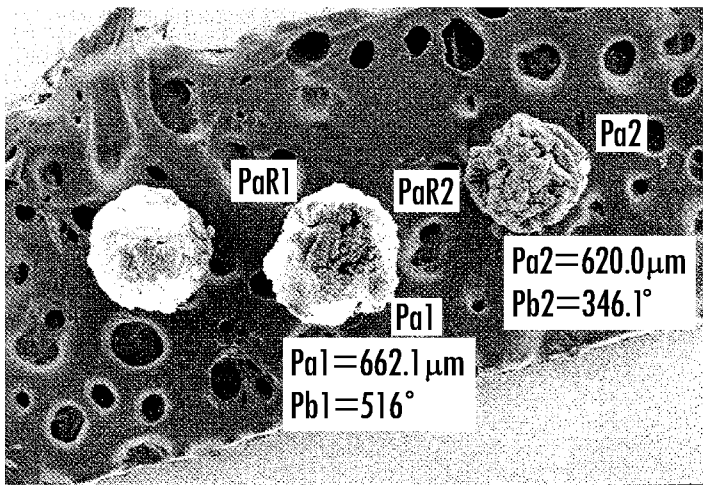
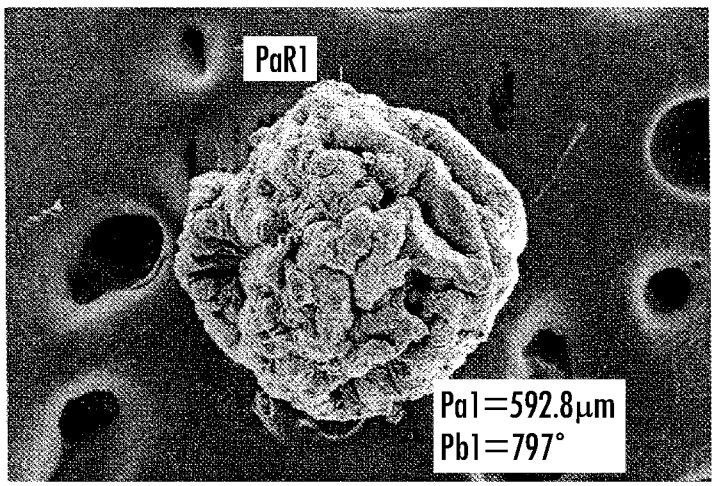

File Name=ptfep1_8.tif 100μm
EHT=10.000kV
Date:16 Jan 2004
Mag=100X
Photo No.=3280
Time:8:47:17

Fig. 3B



File Name=ptfep2_6.tif 200μm EHT=10.00kV Date:23 Jan 2004
Mag=35X Photo No.=3685 Time:9:08:39

Fig. 4A



File Name=ptfep1_9.tif 100μm EHT=10.00kV Signal A=SE2 Date:23 Jan 2004
Mag=100X WD=5mm Photo No.=3678 Time:8:58:29

Fig. 4B

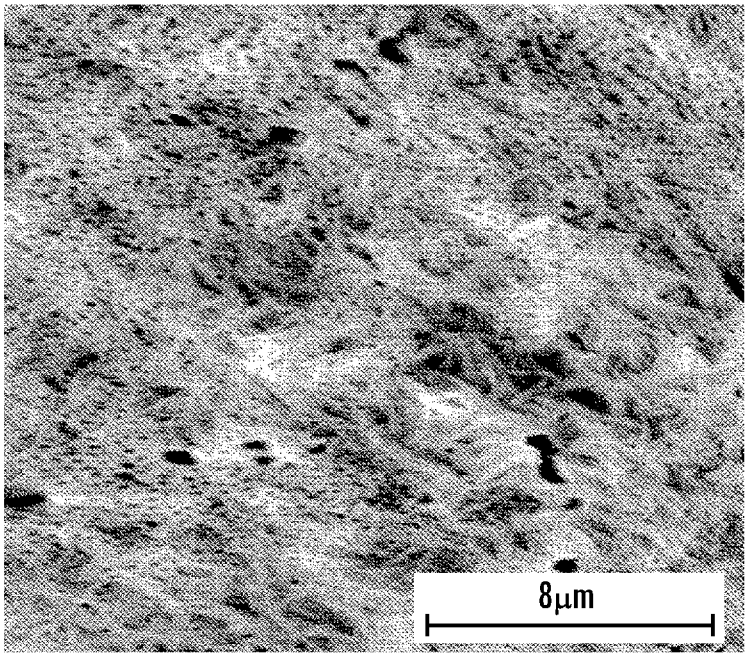


Fig. 5A

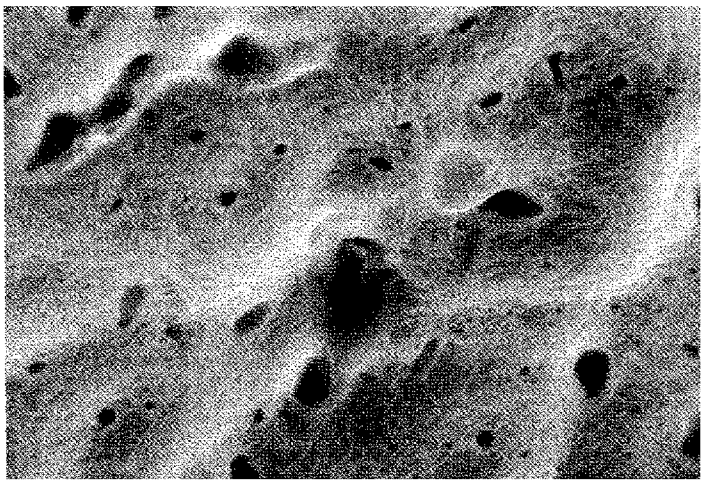


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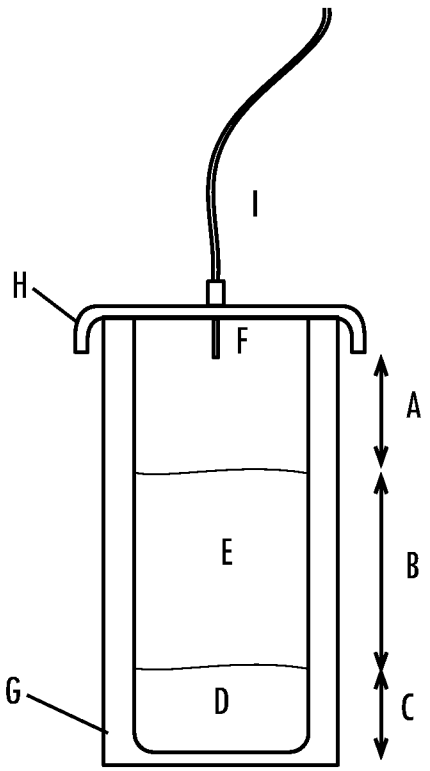
Fig. 5B

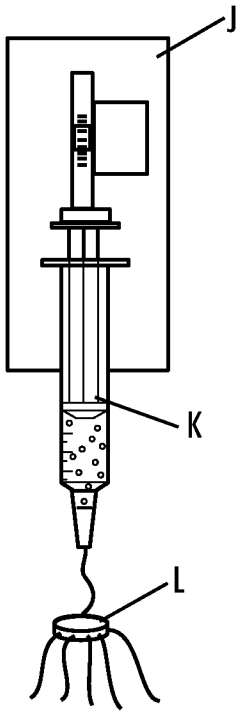
Experimental cryoextraction setup



Legend		Typical dimensions
A	Drop distance length	5-10 cm
B	Liq. Nitrogen layer depth	5-10 cm
C	Nonsolvent layer depth	1-2 cm
D	Nonsolvent	
E	Solvent	
F	Syringe needle tip	(25G-33G)
G	Dewar	(1-21 volume)
H	Lid	
I	Teflon tubing	(0.8mm dia.;40cm length)

Cryovessel

Fig. 6



Legend	
J	Pump housing
K	Syringe
L	Teflon distributor with Teflon tubing attached

Syringe Pump

Fig. 7

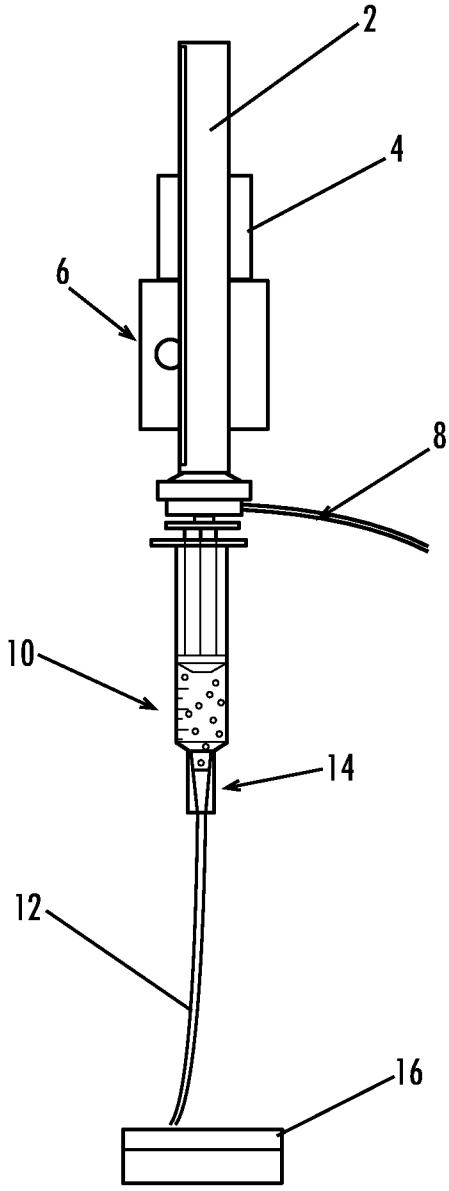
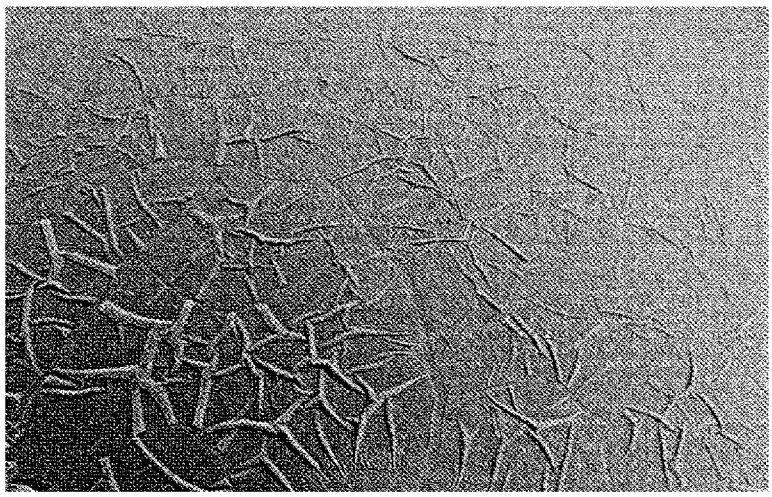
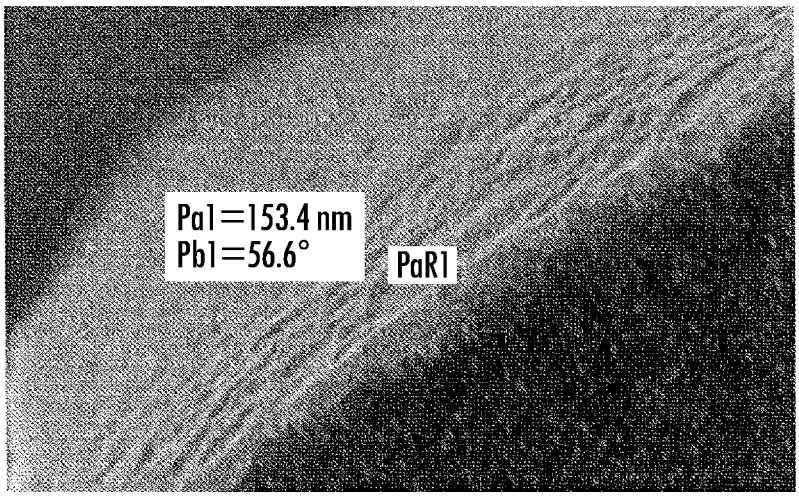


Fig. 8



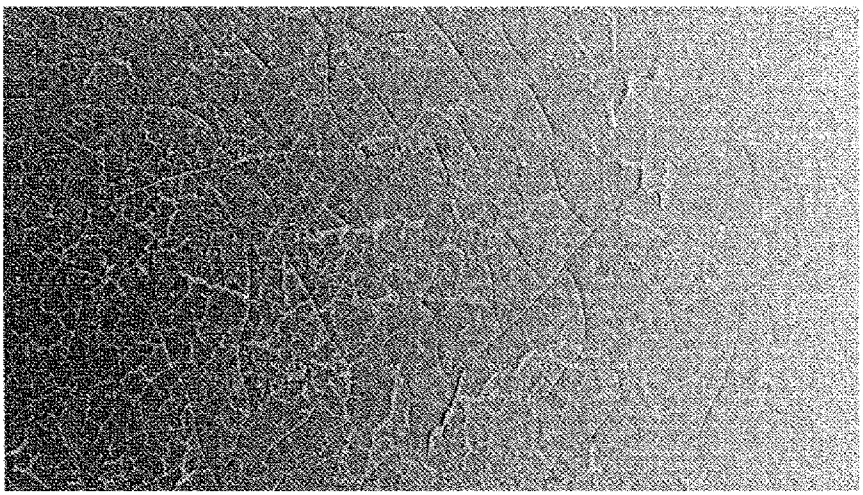
10μm EHT=3.00kV Signal A=SE2 Date:21 Apr 2006
Mag=1.00kX WD=5mm Photo No.=7212 Time:8:38:21

Fig. 9A



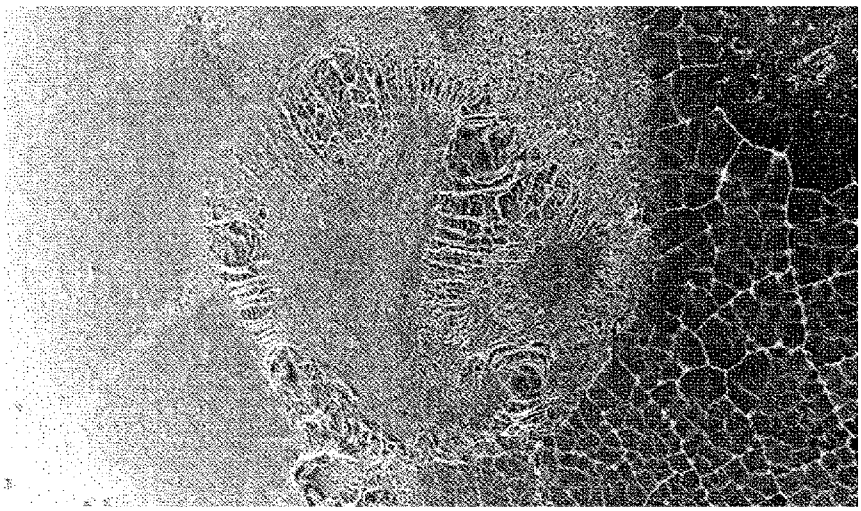
100 nm EHT=3.00kV Signal A=SE2 Date:21 Apr 2005
Mag=50.00kX WD=4mm Photo No.=7258 Time:9:20:13

Fig. 9B



10μm EHT=3.00kV Signal A=SE2 Date:21 Apr 2005
Mag=1.00kX WD=5mm Photo No.=7293 Time:9:55:52

Fig. 10A



10μm EHT=3.00kV Signal A=InLens Date:21 Apr 2006
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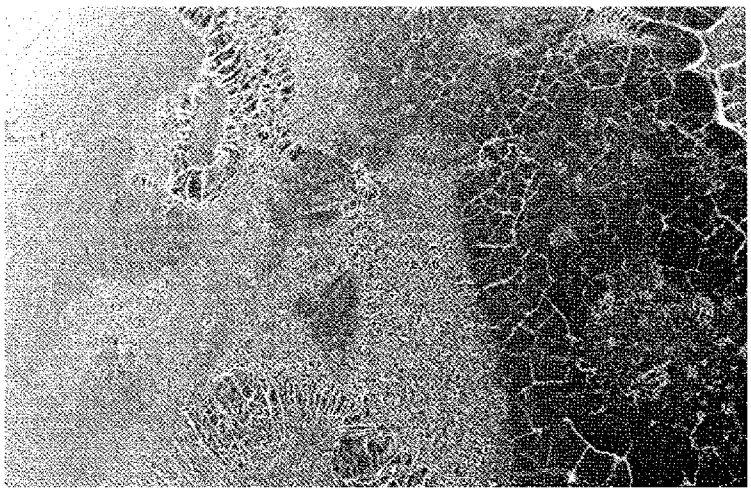
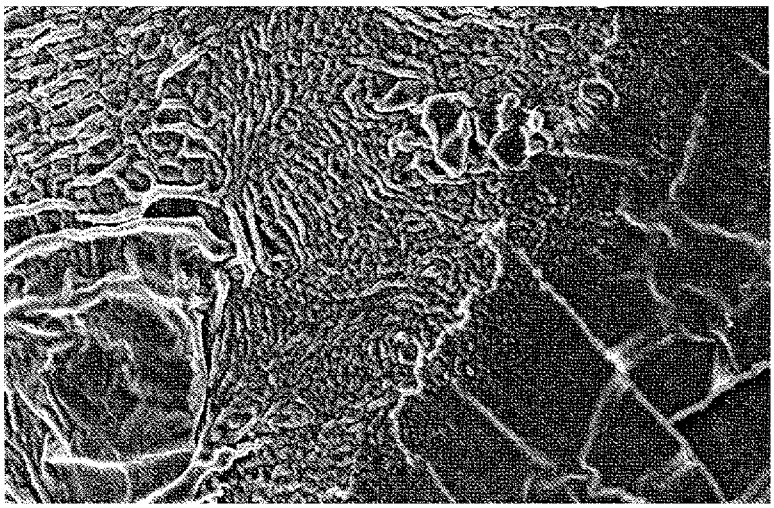
Fig. 10B



10μm EHT=3.00kV Signal A=InLens Date:21 Jan 2005
Mag=1.00kX WD=5mm Photo No.=7296 Time:9:58:54

Fig. 10C



2μm EHT=3.00kV Signal A=InLens Date:21 Apr 2005
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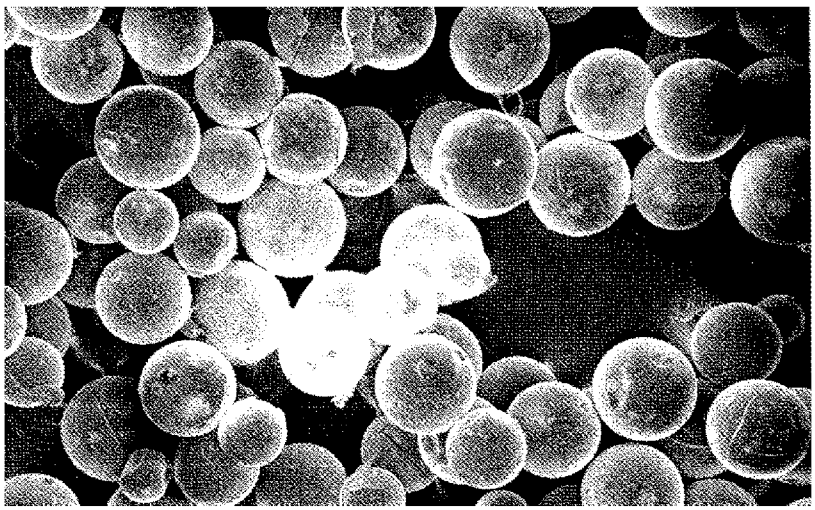
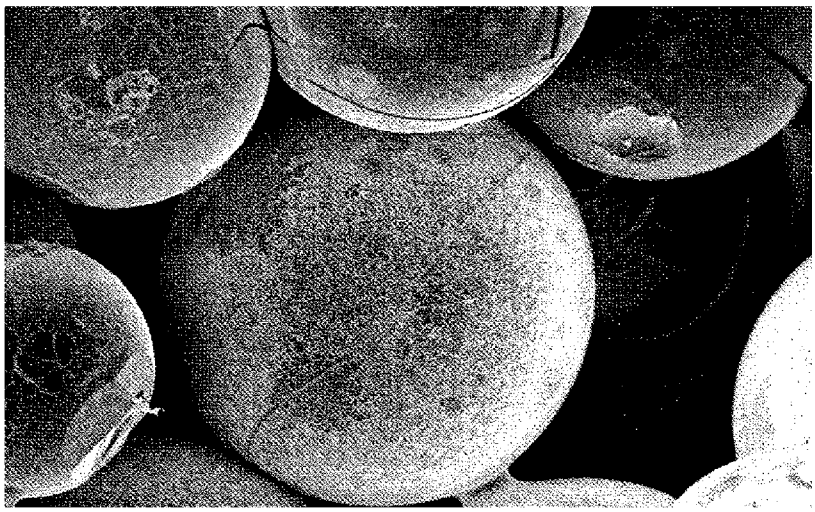
Fig. 10D



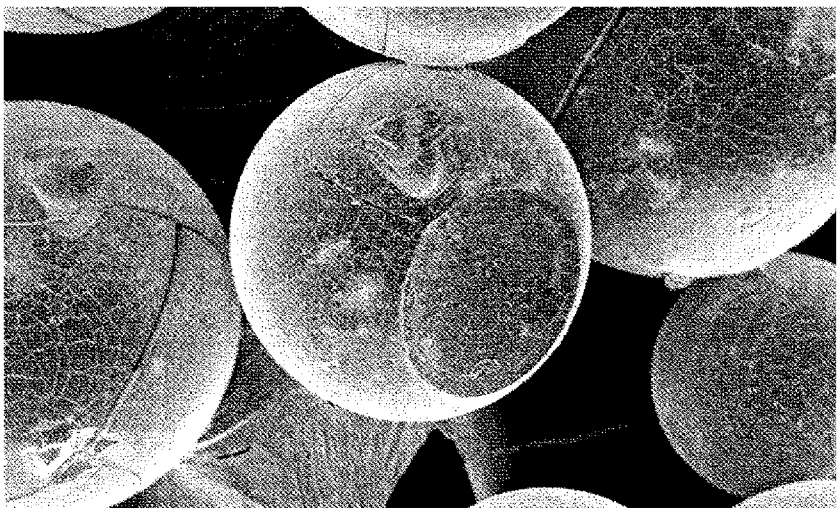
100μm EHT=3.00kV Signal A=InLens Date:21 Apr 2005
Mag=50X WD=5mm Photo No.=7269 Time:9:37:22

Fig. 11A



100μm EHT=3.00kV Signal A=InLens Date:21 Apr 2005
Mag=5.00kX WD=5mm Photo No.=7270 Time:9:38:43

Fig. 11B



30μm EHT=3.00kV Signal A=InLens Date:21 Apr 2005
Mag=200X WD=5mm Photo No.=7269 Time:9:33:46

Fig. 11C

10μm EHT=3.00kV Signal A=InLens Date:21 Apr 2005
Mag=1.00kX WD=5mm Photo No.=7262 Time:9:28:51

Fig. 11D

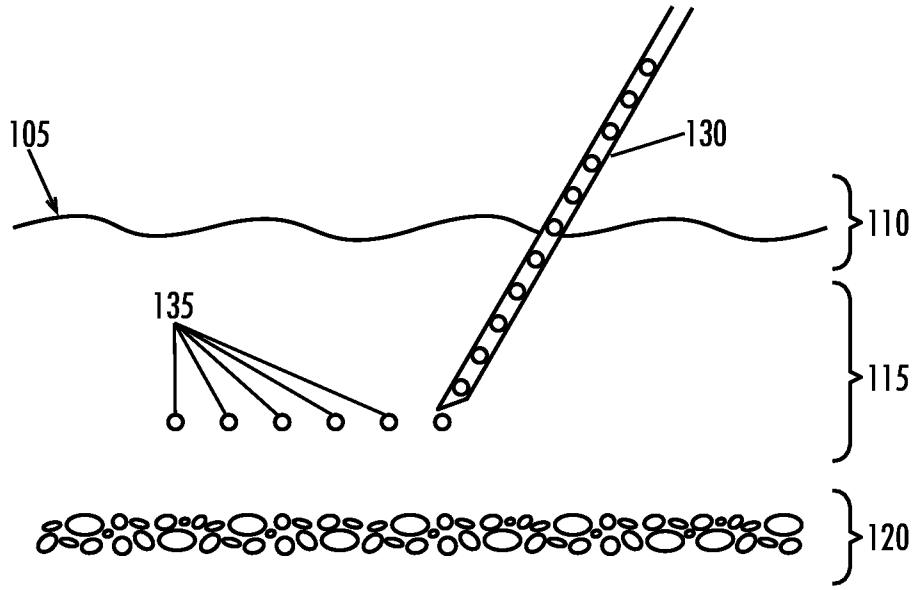


Fig. 12

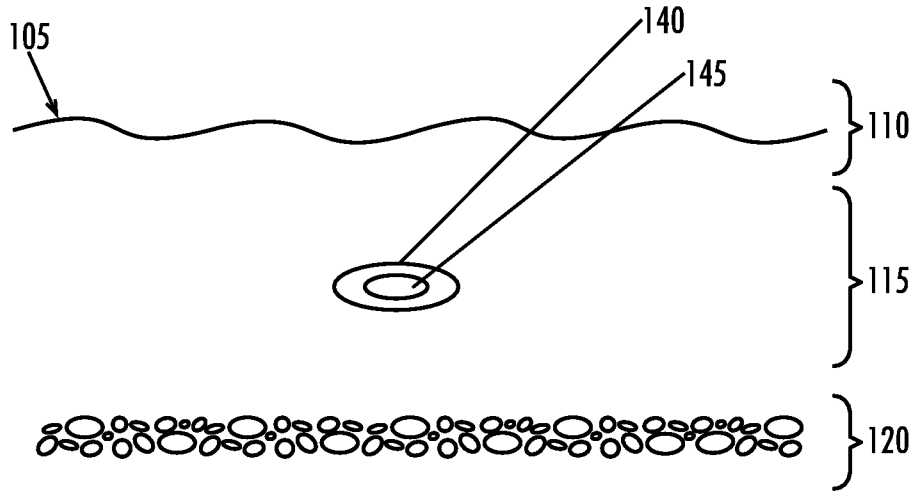


Fig. 13

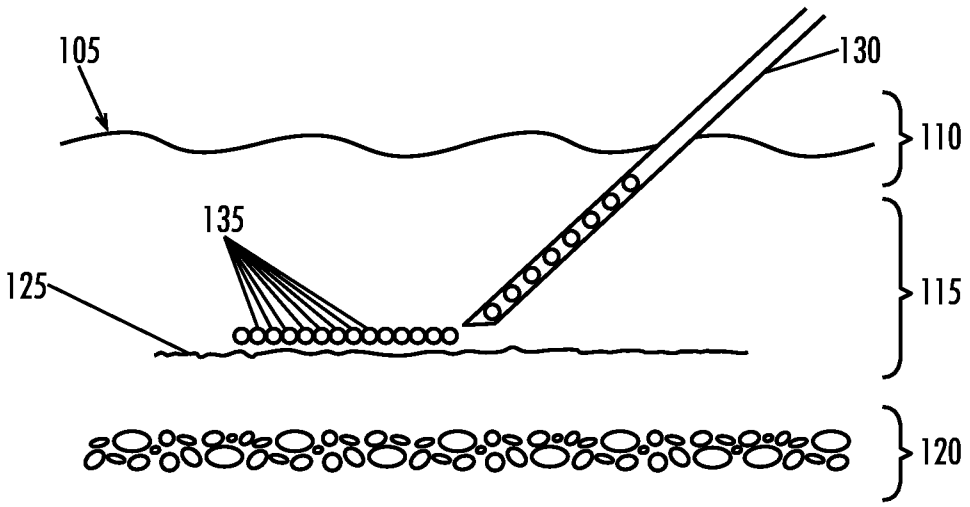


Fig. 14

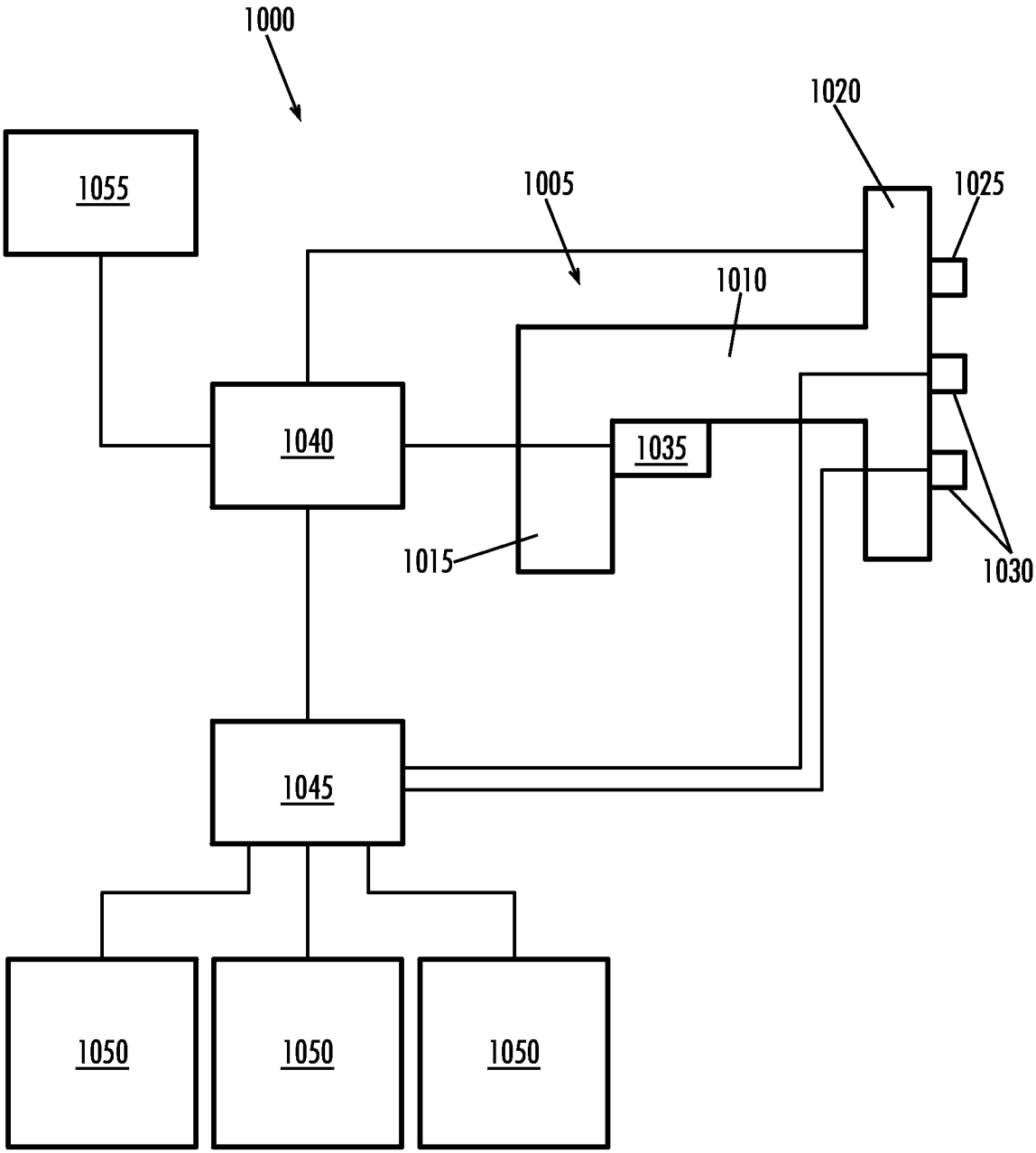

1000
1020
1055
1005
1025
1010
1040
1035
1015
1030
1045
1050
1050
1050

Fig. 15

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/083043

A. CLASSIFICATION OF SUBJECT MATTER
INV. C09D185/02

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)
C08L C09D C08J

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 practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/046155 A (POLYZENIX GMBH [DE]; HARDER PHILLIP [DE]; FRITZ OLAF [DE]; FRITZ ULF [] 4 May 2006 (2006-05-04) page 16, line 19; claims	1-9
A	CHAMPION ET AL: "Particle shape: A new design parameter for micro- and nanoscale drug delivery carriers" JOURNAL OF CONTROLLED RELEASE, ELSEVIER, AMSTERDAM, NL, vol. 121, no. 1-2, 31 July 2007 (2007-07-31), pages 3-9, XP022179901 ISSN: 0168-3659 the whole document	1

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"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)

"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

"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

"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

"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.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 August 2008	27/08/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Deraedt, Gilbert

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/083043

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2006/201673 A1 (WELTON THOMAS D [US] ET AL) 14 September 2006 (2006-09-14) claims	1
A	WO 01/70296 A (POLYZENIX GMBH [DE]; GRUNZE MICHAEL [DE]) 27 September 2001 (2001-09-27) claims	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/083043

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 10-25
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 10-25 do deal with methods practised on the human/animal body.(Rule 39, 1, iv PCT)

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2007/083043

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2003099683 A1	29-05-2003