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(54) **DELIVERY SYSTEM FOR DRUG AND CELL THERAPY**

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A61K 9/127	(2006.01)
A61K 38/02	(2006.01)
A61K 9/48	(2006.01)
A61K 35/12	(2006.01)
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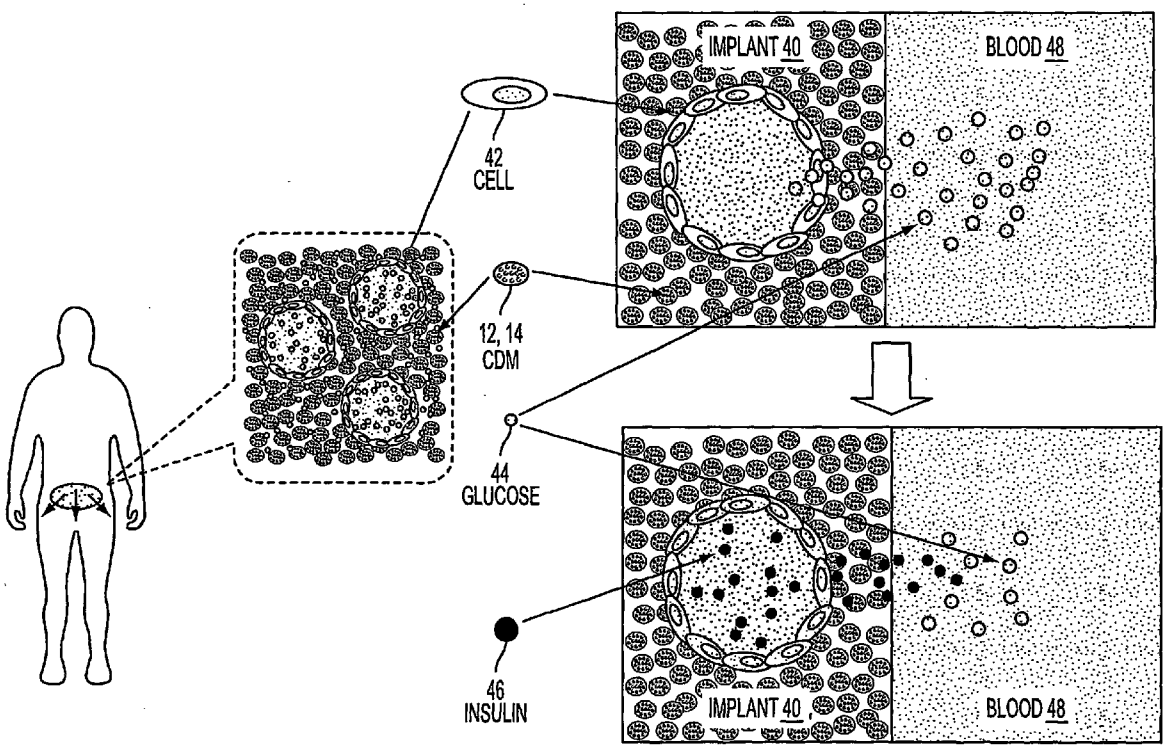
A61M 15/00	(2006.01)

(52) **U.S. Cl.** ..... **424/450**; 424/490; 424/497; 424/493; 514/44 R; 424/93.6; 424/93.1; 514/5.9; 514/1.1; 424/451; 536/112; 128/203.15

(57) **ABSTRACT**

A method of lowering blood glucose in a mammal includes administering orally or by injection or inhalation a therapeutically effective amount of crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal. The composition may be a one phase or a structured multi-phase composition for controlled release of insulin or other therapeutic agents.



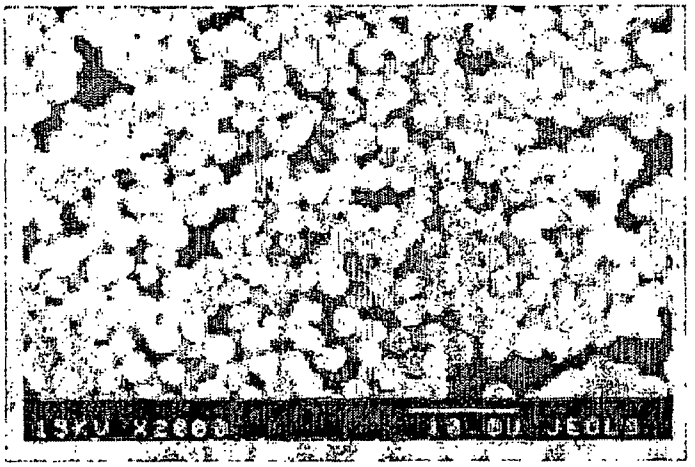


FIG. 1

FIG. 2A

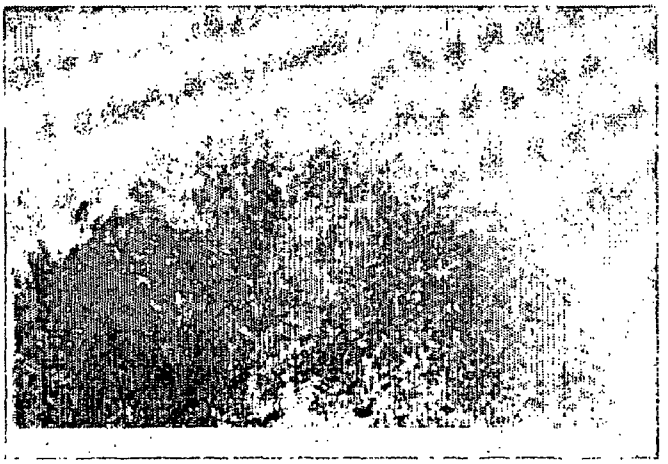


FIG. 2B

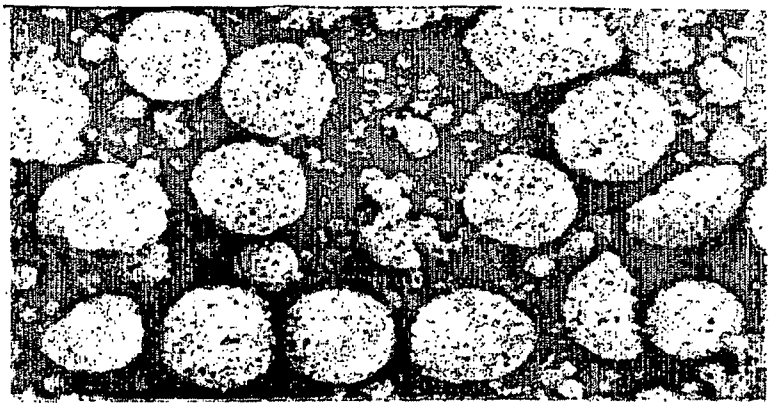


FIG. 3

FIG. 4

FIG. 5

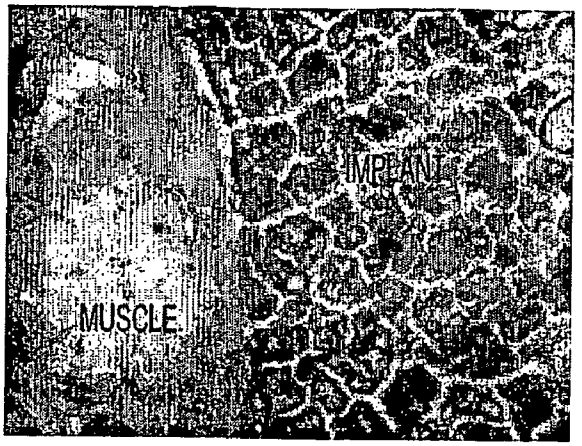


FIG. 6A

FIG. 6B

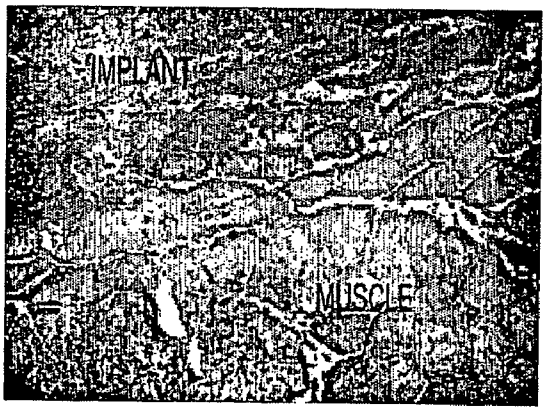


FIG. 6C

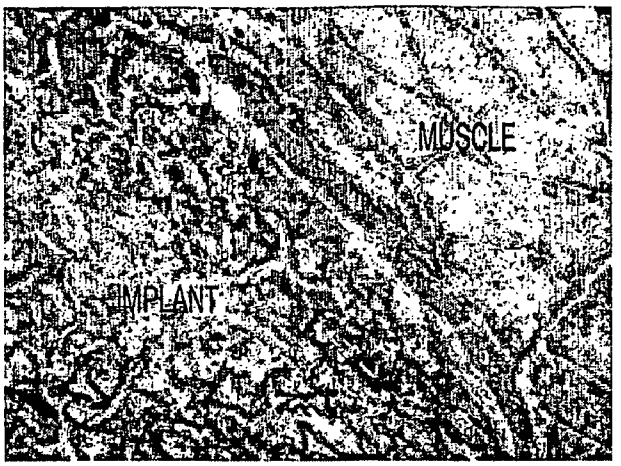


FIG. 7A

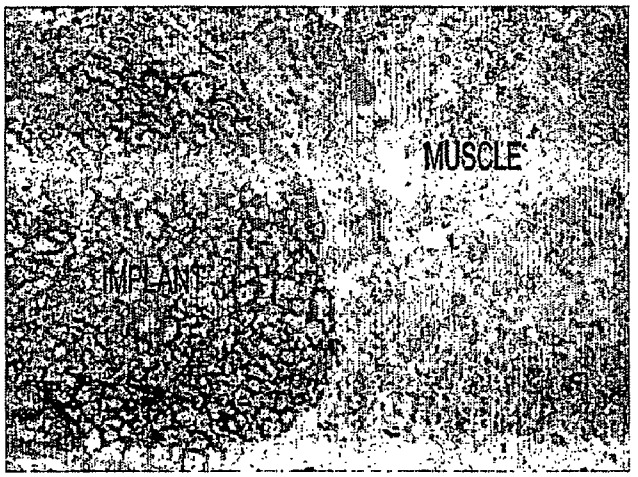


FIG. 7B

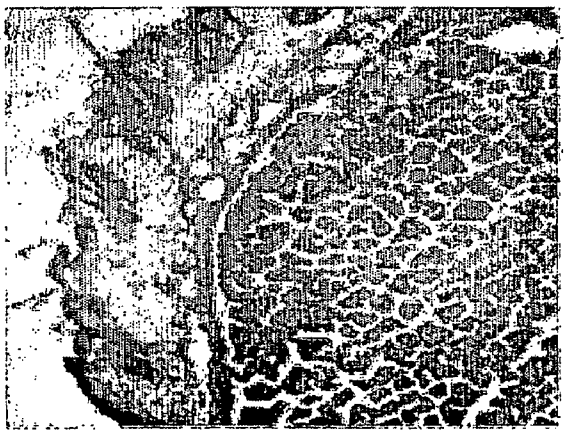


FIG. 8A

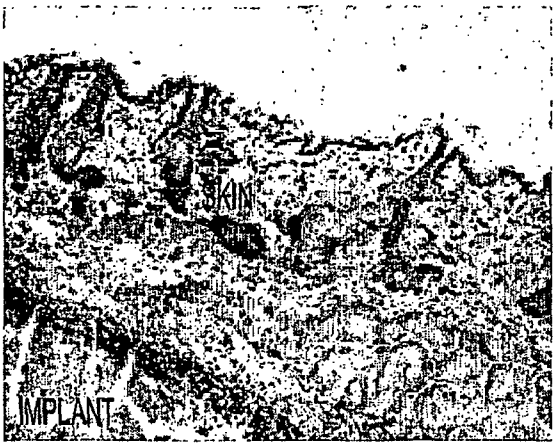


FIG. 8B

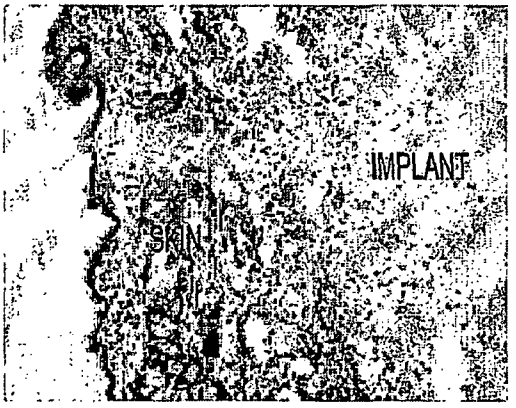


FIG. 8C

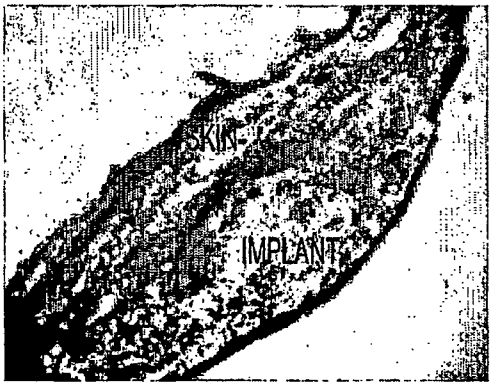


FIG. 8D

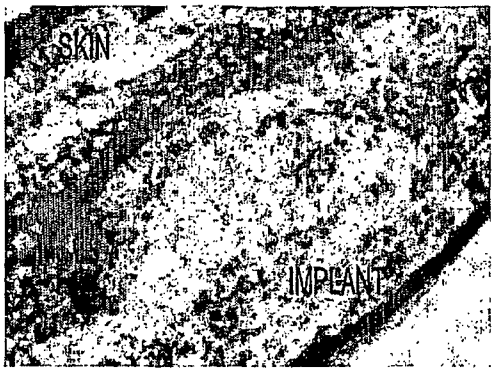


FIG. 8E

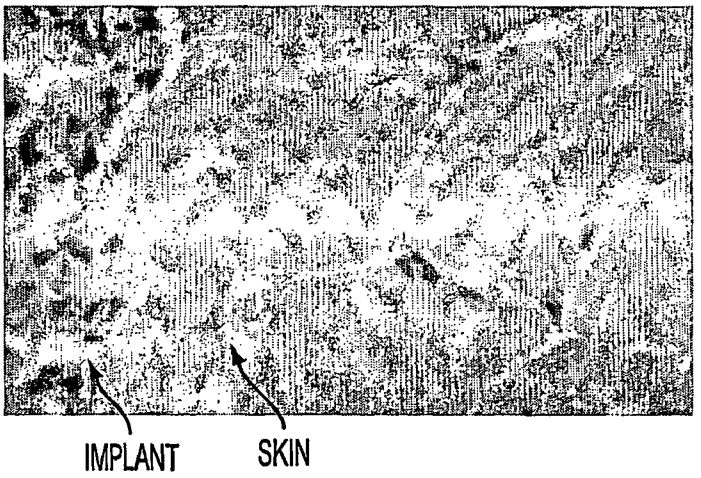


FIG. 8F

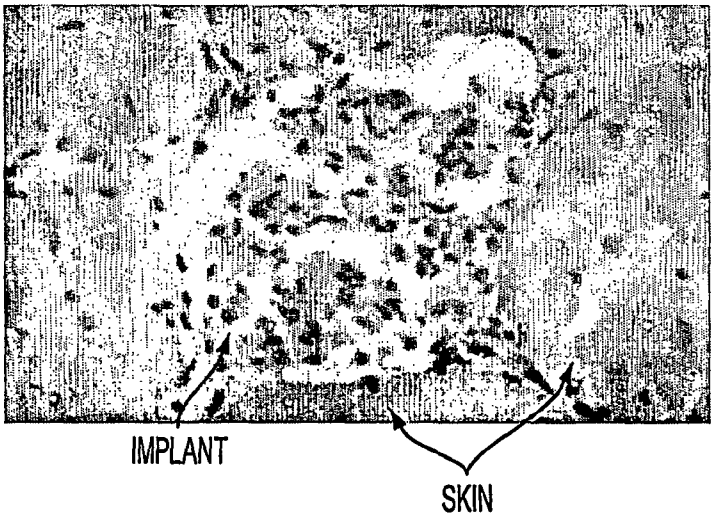


FIG. 8G

FIG. 9

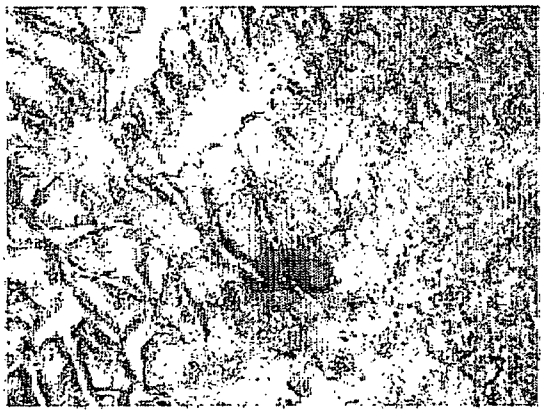


FIG. 10

FIG. 11

FIG. 12

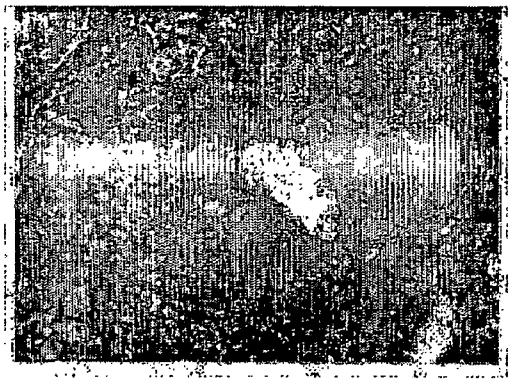


FIG. 13

FIG. 14

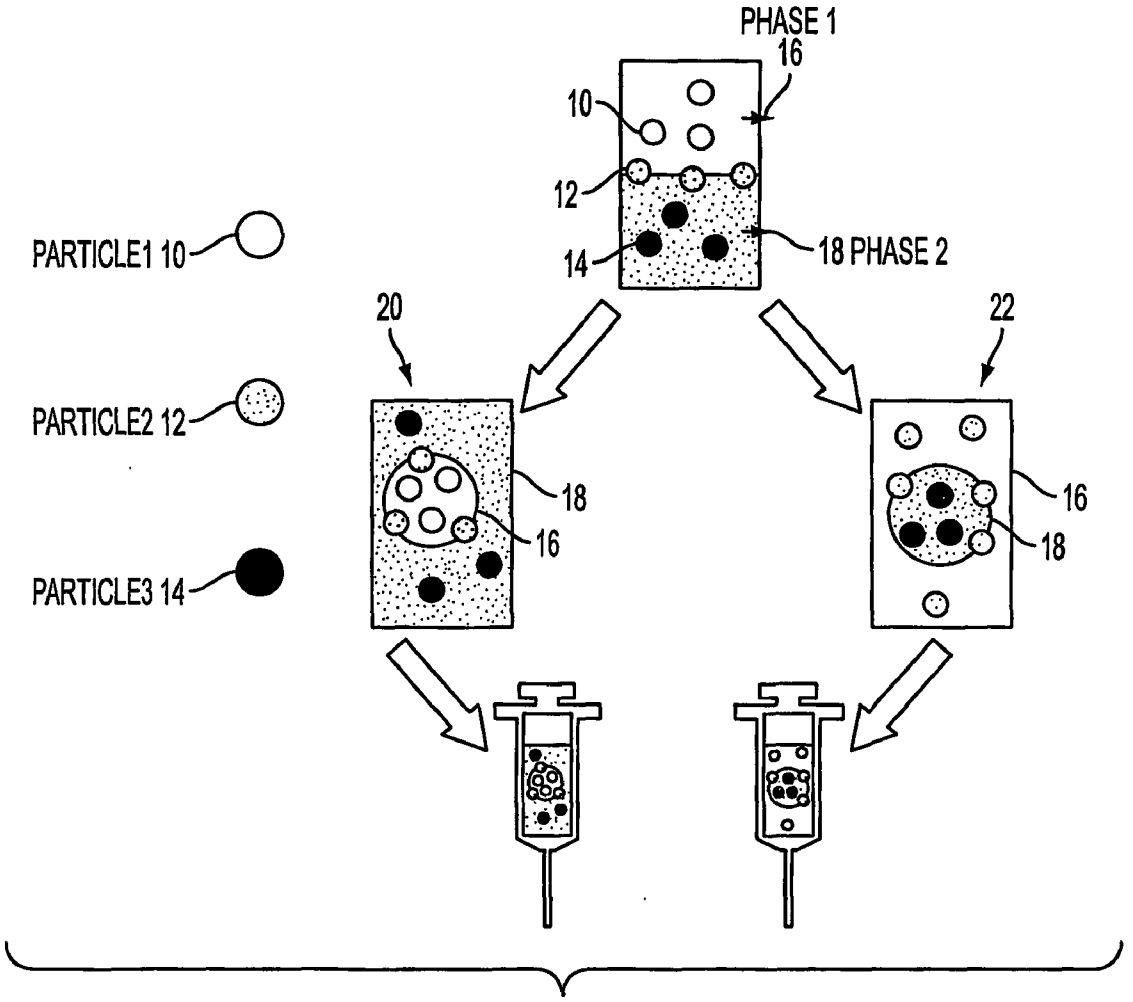

PHASE 1
16
10
12
14
18 PHASE 2
PARTICLE1 10
PARTICLE2 12
PARTICLE3 14
20
22
18
16
16
18

FIG. 15A

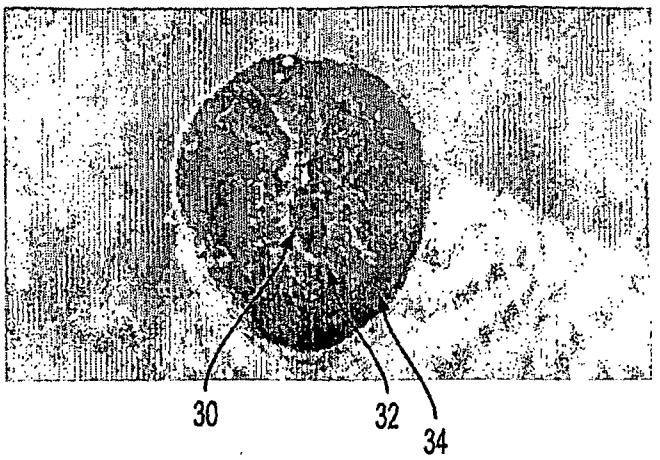


FIG. 15B

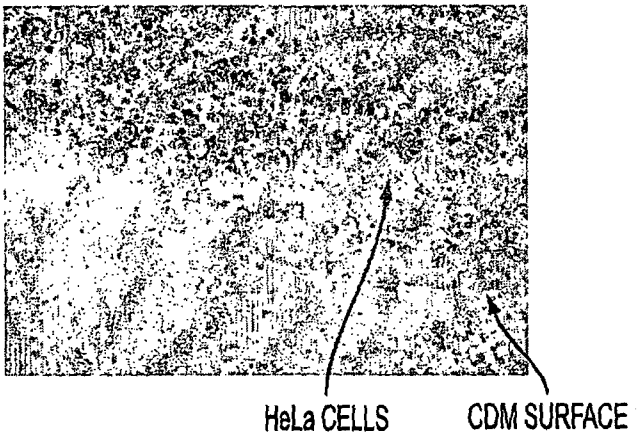


FIG. 16A

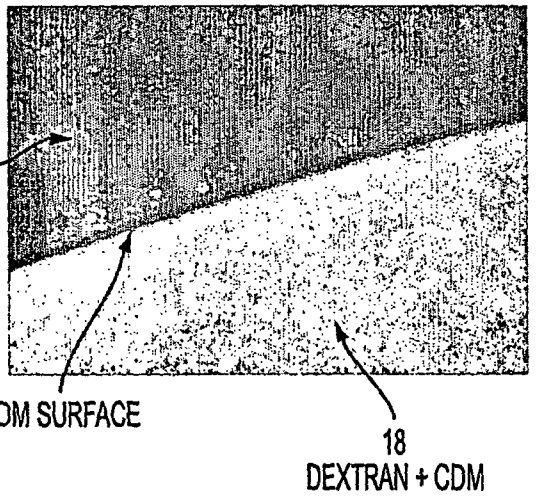


FIG. 16B

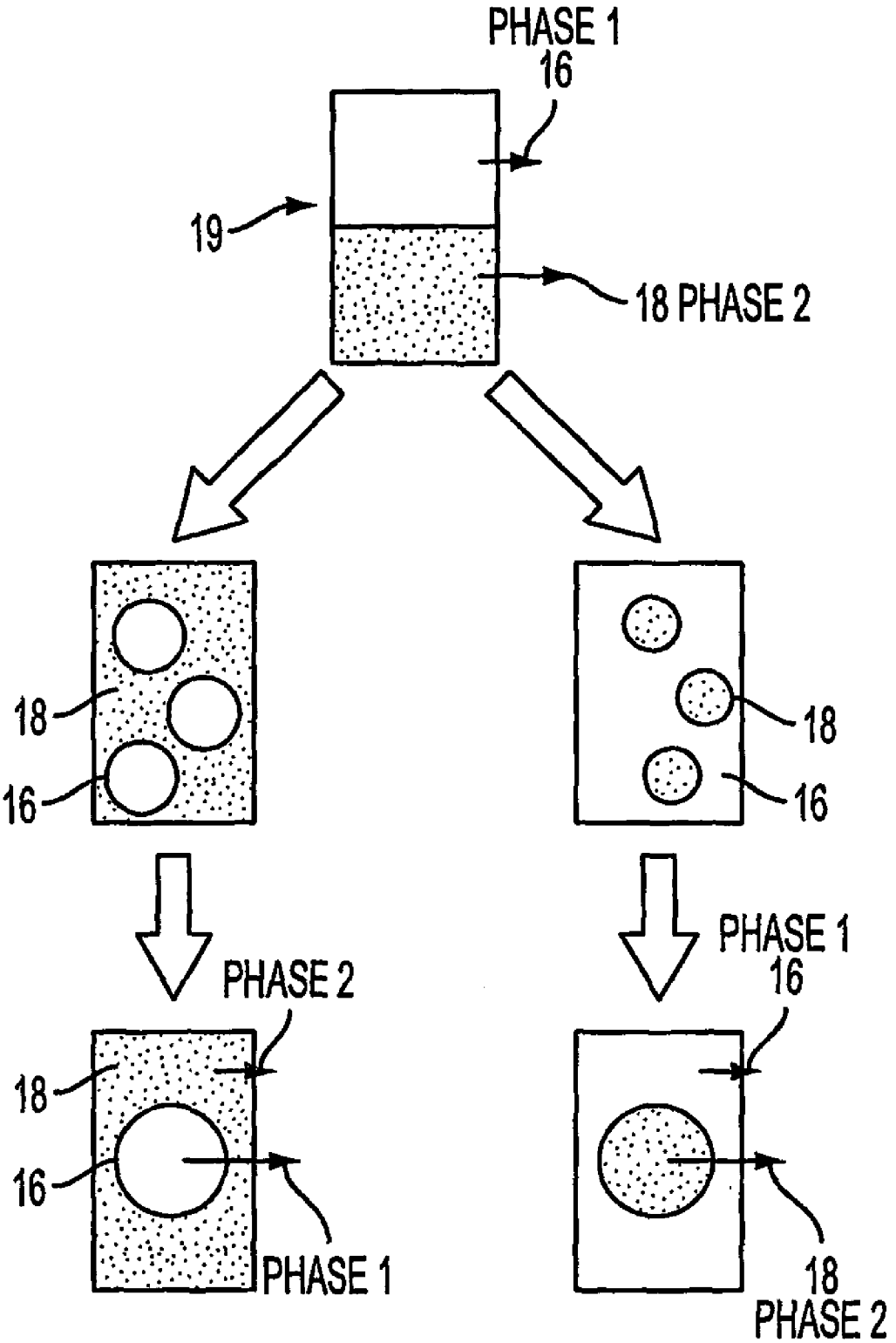

PHASE 1
16
19
18 PHASE 2
18
16
18
16
PHASE 2
PHASE 1
16
18
16
PHASE 1
18
PHASE 2

FIG. 15C

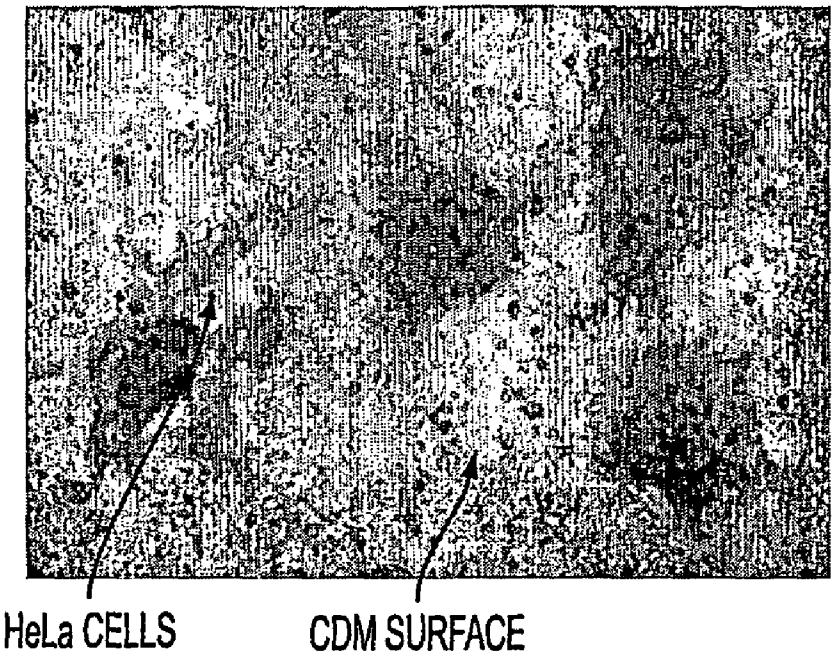


FIG. 16C

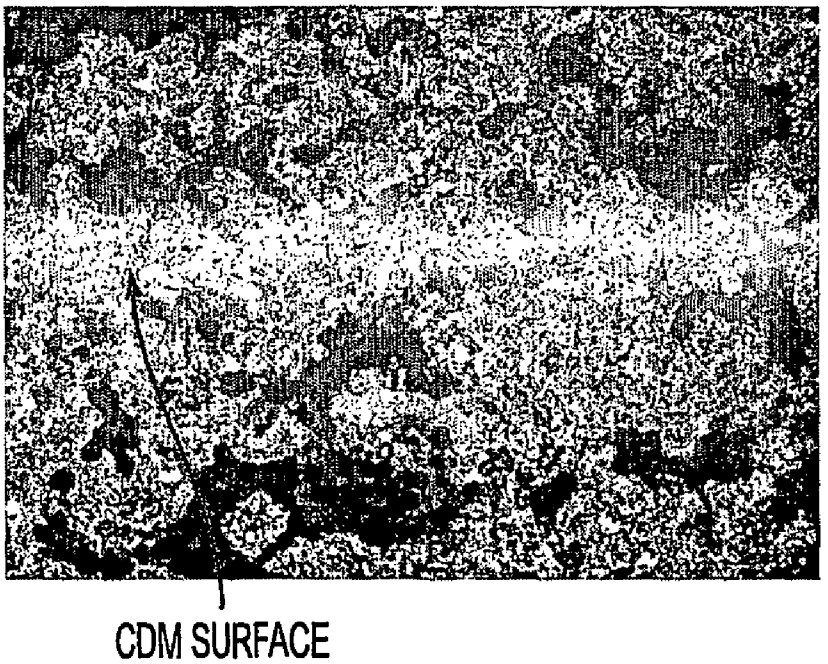


FIG. 16D

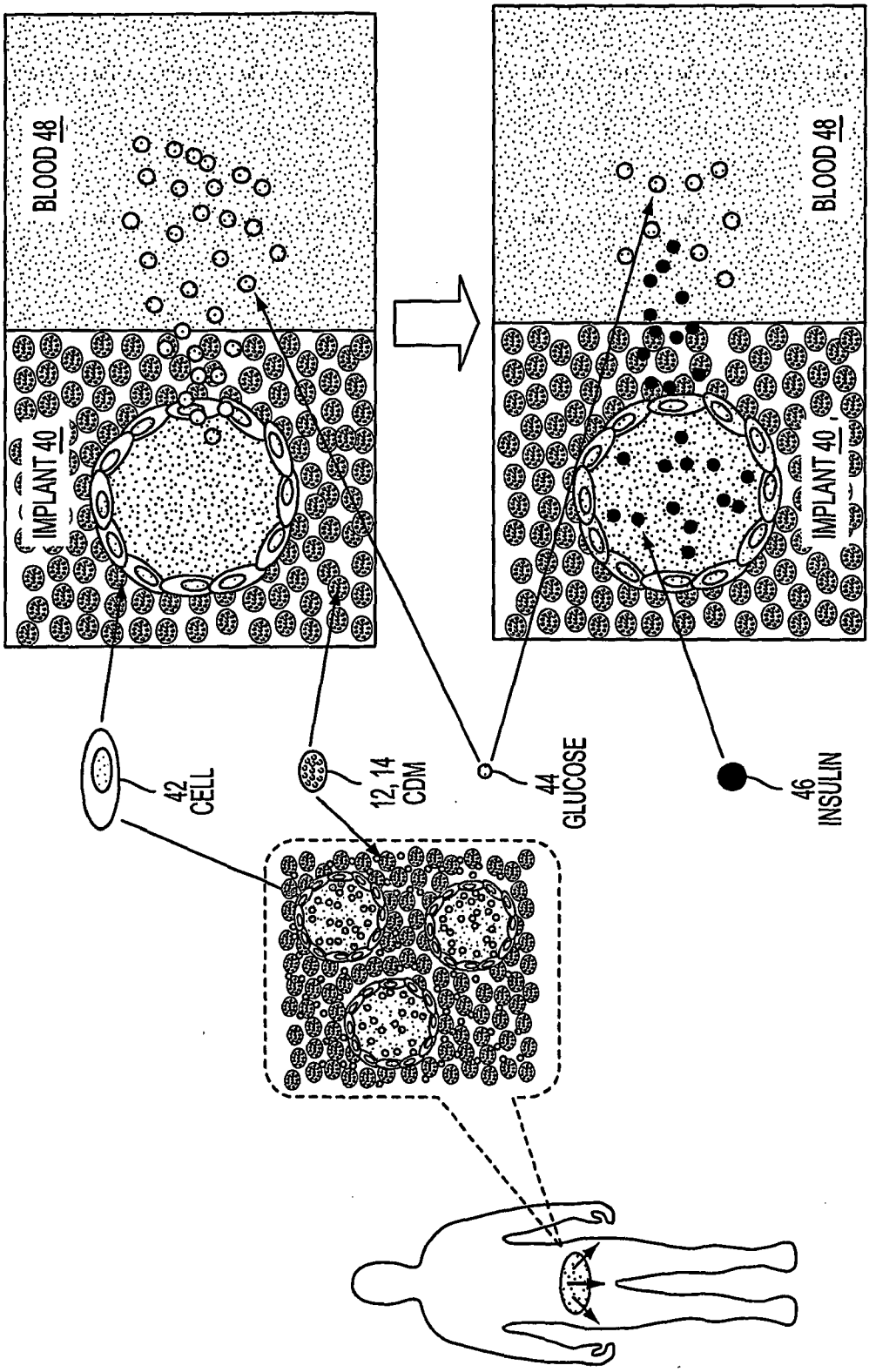

BLOOD 48
IMPLANT 40
BLOOD 48
IMPLANT 40
42
CELL
12, 14
CDM
44
GLUCOSE
46
INSULIN

FIG. 17

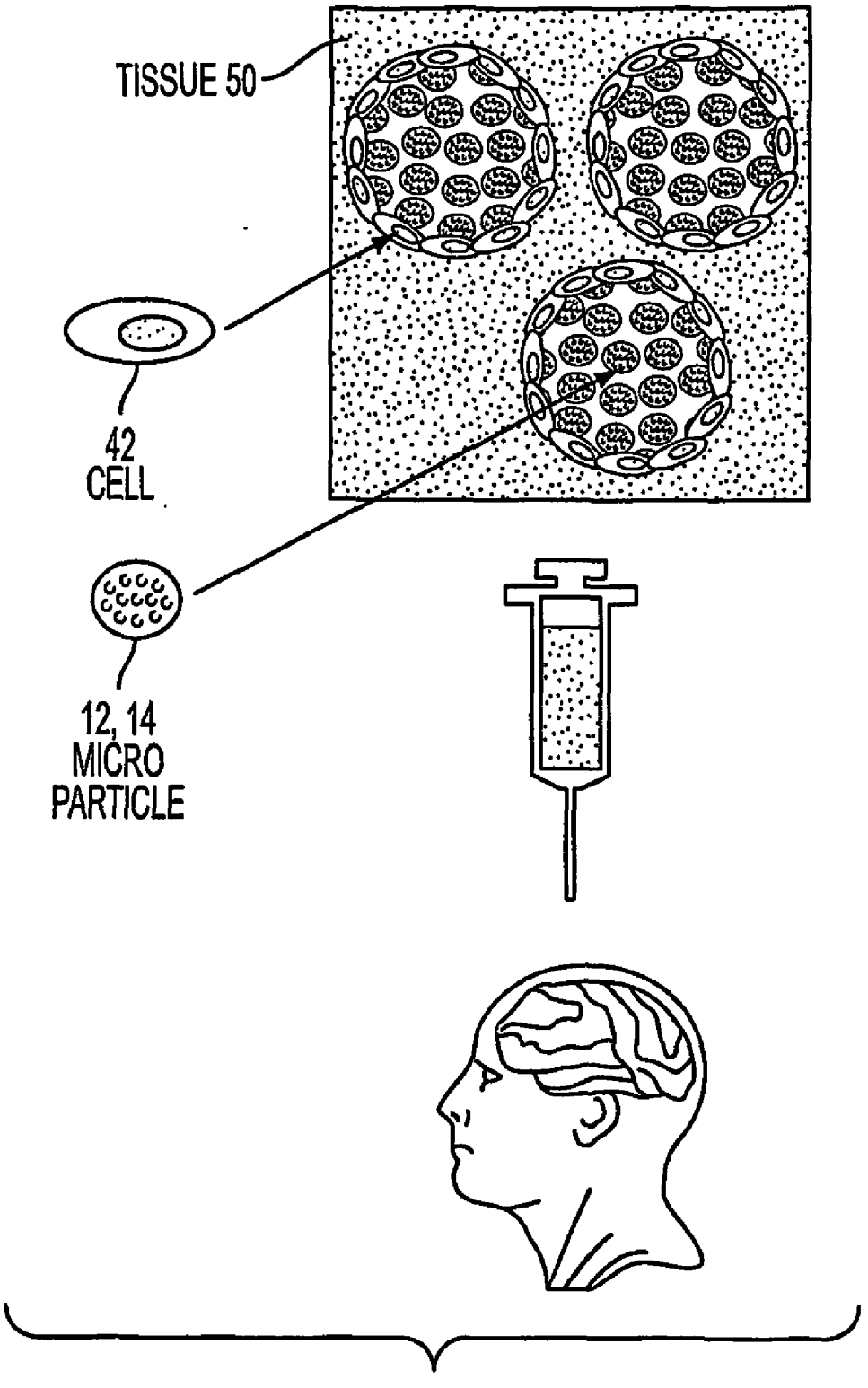


FIG. 18

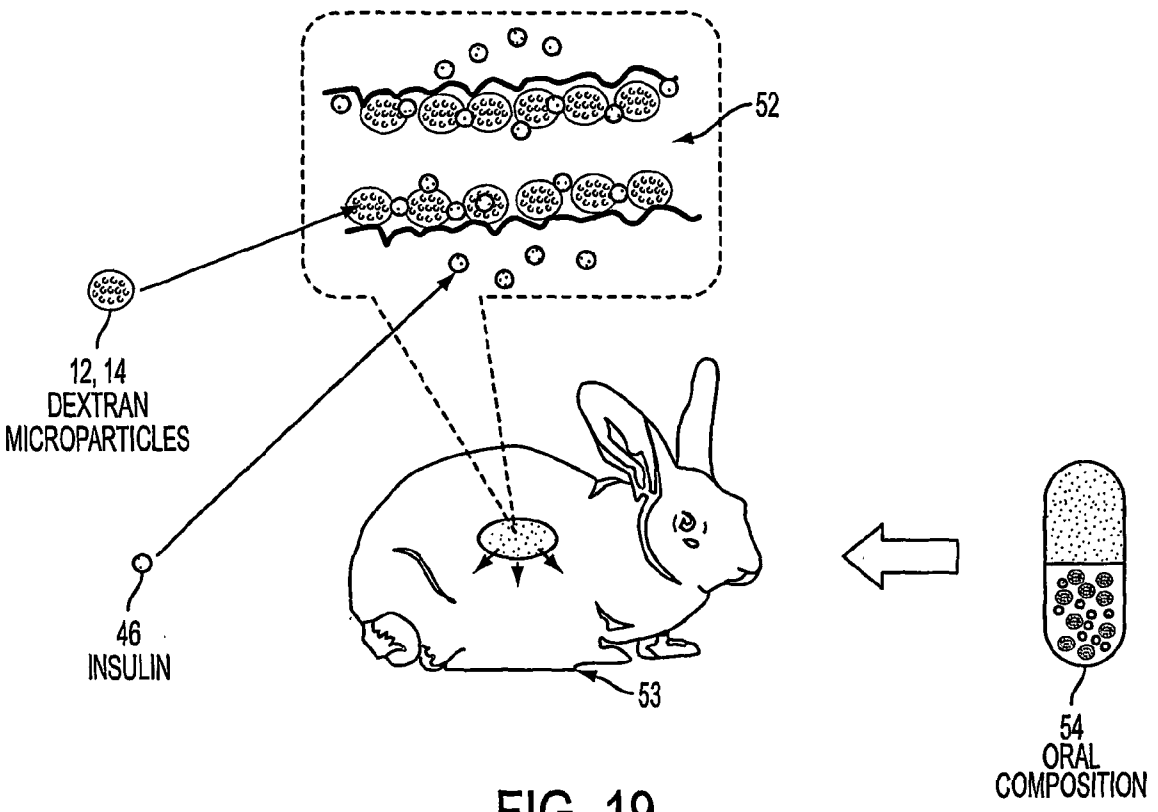

52
12, 14
DEXTRAN
MICROPARTICLES
46
INSULIN
53
54
ORAL
COMPOSITION

FIG. 19

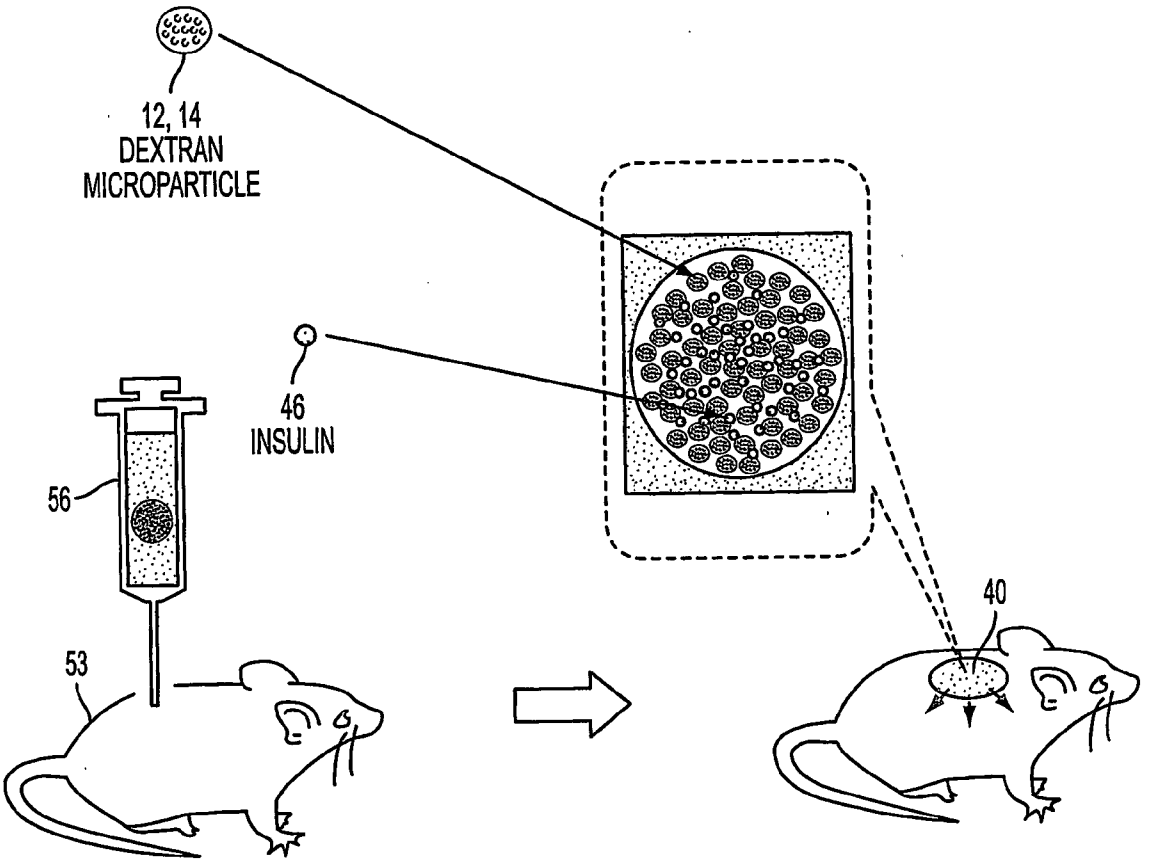


FIG. 20

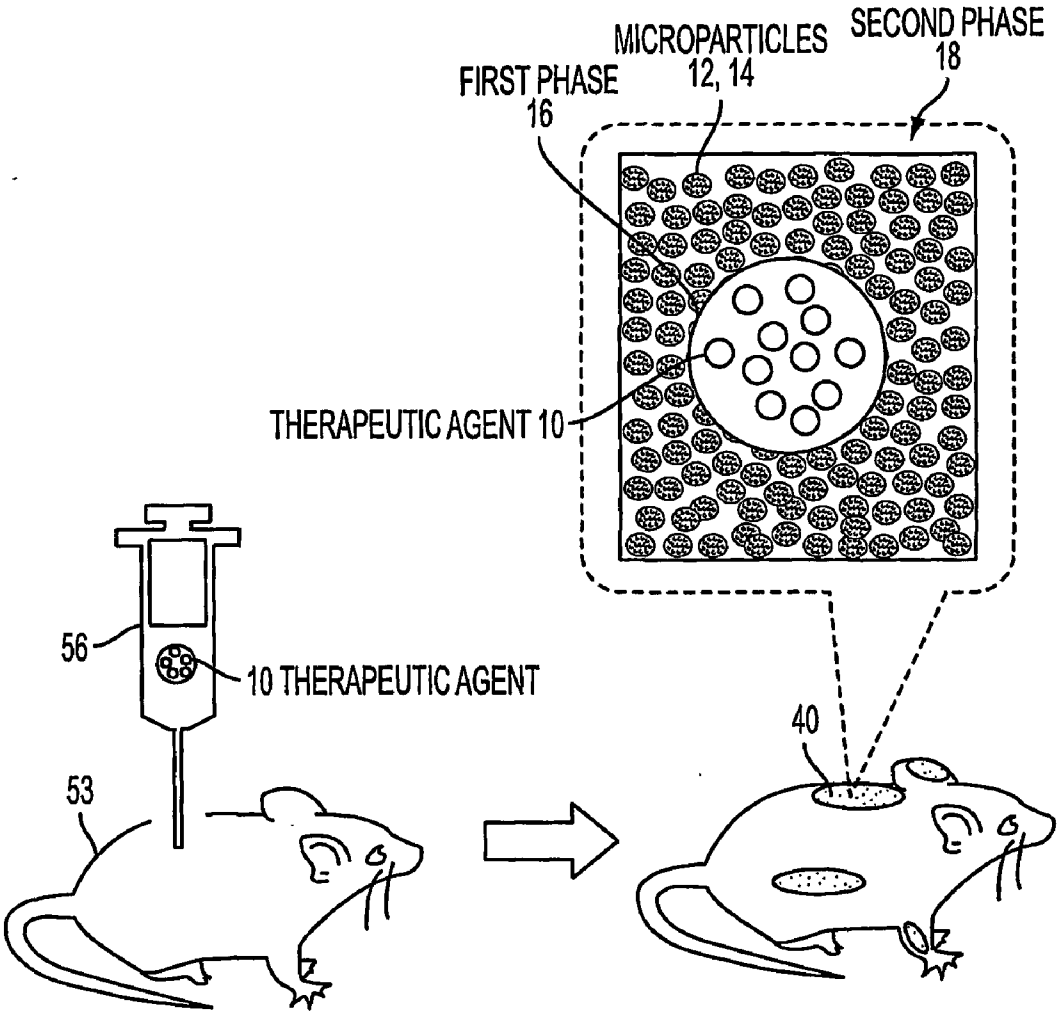

MICROPARTICLES
12, 14
SECOND PHASE
18
FIRST PHASE
16
THERAPEUTIC AGENT 10
56
10 THERAPEUTIC AGENT
53
40

FIG. 21

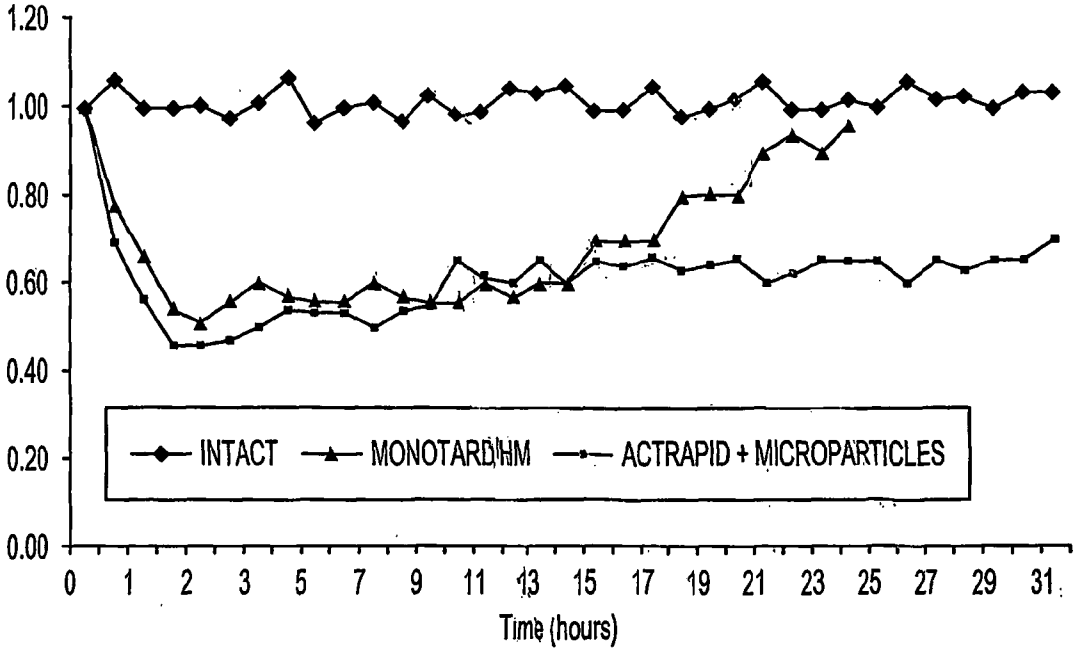


FIG. 22A

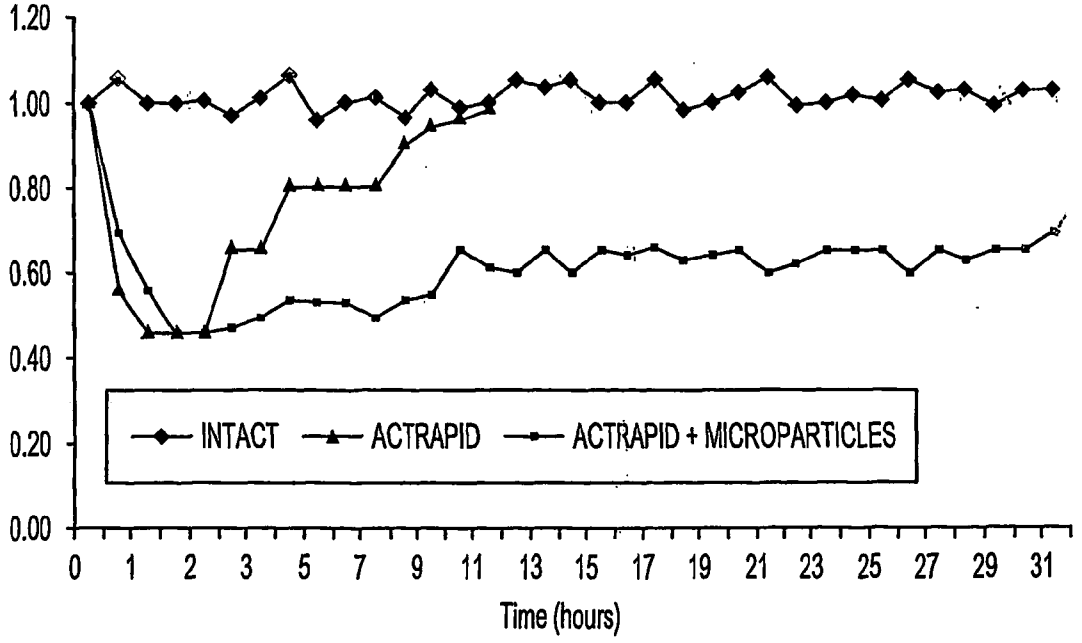


FIG. 22B

DELIVERY SYSTEM FOR DRUG AND CELL THERAPY

[0001] This application claims benefit of the following U.S. Provisional Application Ser. Nos. 60/451,245, filed Mar. 4, 2003; 60/467,601 filed May 5, 2003; 60/469,017 filed May 9, 2003; and 60/495,097 filed Aug. 15, 2003, the disclosures of which are incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates generally to biomaterials development for systemic and local delivery of therapeutic agents directly within or upon body tissues and for tissue engineering. In particular, the present invention is directed to the fabrication of biocompatible implants which contain cells, biologically active substances or combinations thereof and to microparticle matrix materials for delivery of proteins, peptides and nucleic acids.

BACKGROUND OF THE INVENTION

[0003] The term "biomaterial" has alternately been used to describe material derived from biological sources or to describe material used for therapies in the human body. The present invention is related to the latter one. The term "biocompatible" is used herein to mean that the material by itself or in combination with therapeutic agents, including living cells, does not produce foreign body reaction or fibrosis.

[0004] Dextrans are high molecular weight polysaccharides synthesized by some micro organisms or by biochemical methods. Dextran with average molecular weight of about 75 kDa has a colloid osmotic pressure similar to blood plasma, so its aqueous solutions are used clinically as plasma expanders. Cross-linked dextrans in the form of beads are the basis for "Sephadex®" that is used in the GPC of proteins and for "Cytodex®" developed by Pharmacia to fulfill the special requirements of a micro-carrier cell culture. For example, U.S. Pat. Nos. 6,395,302 and 6,303,148 (Hennink et al.) disclose attaching various biomaterials to cross-linked dextran particles. However, beads based on cross-linked dextran generally cannot be used for implant manufacturing owing to their potential toxicity due to the application of cross-linking agents (Blain J. F., Maghni K., Pelletier S, and Sirois P. Inflamm. Res. 48 (1999): 386-392).

[0005] U.S. Pat. No. 4,713,249 (Schroder) describes a method of producing a depot matrix for biologically active substances. According to this patent, the depot matrix allegedly consists of carbohydrate microparticles, stabilized by crystallization, which implies using non-covalent bonds. The following process for producing the alleged crystallized carbohydrate microparticles is described by Schroder. A solution of a polymeric carbohydrate and a biologically-active substance is formed in one or more hydrophilic solvents. Then the mixture of the carbohydrate and the biologically active substance is emulsified in a liquid hydrophobic medium to form spherical droplets. The emulsion is then introduced into a crystallizing medium comprising acetone, ethanol or methanol to form spheres having a non-covalently cross-linked crystalline polymeric carbohydrate matrix, said matrix incorporating 0.001-50% by weight of the biologically-active substance. Thus, the biologically active substance is provided into the solution prior to crystallizing the spheres. Schroder does not describe the microstructure of the microparticles made by the multi-step method. Schroder's multi-step method is complex and uses organic solvents that are potentially toxic to cells and need to be removed.

BRIEF SUMMARY OF THE INVENTION

[0006] A method of lowering blood glucose in a mammal includes administering orally or by injection or inhalation a therapeutically effective amount of crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal. The composition may be a one phase or a structured multi-phase composition for controlled release of insulin or other therapeutic agents

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. **1** is a photograph of crystallized dextran microparticles spontaneously formed in 55.0% (W/W) aqueous solution of dextran with MW 70.0 kDa.

[0008] FIG. **2**A is a photograph of a cross-section of crystallized dextran microparticles shown in FIG. **1**.

[0009] FIG. **2**B is a photograph of a cross-section of a microparticle shown in FIG. **2**A. Microporous structure of the microparticle can be seen.

[0010] FIG. **3** is a photograph of aggregates of crystallized dextran microparticles.

[0011] FIG. **4** is a photograph of a subcutaneously injected implant consisting of crystallized dextran microparticles shown by FIG. **3**.

[0012] FIG. **5** is a photograph of an intramuscular injected implant consisting of crystallized dextran microparticles shown by FIG. **3**.

[0013] FIGS. **6**A, **6**B and **6**C are photographs of a cross-section of mouse muscle with injected implant consisting of crystallized dextran microparticles (1^{st}, 4^{th}, and 28^{th} day after injection, respectively).

[0014] FIGS. **7**A and **7**B are photographs of a cross-section of mouse muscle with injected implant consisting of crystallized dextran microparticles (180 days after injection).

[0015] FIGS. **8**A, **8**B, **8**C, **8**D, **8**E, **8**F and **8**G are photographs of a cross-section of mouse skin with injected implant consisting of crystallized dextran microparticles (1^{st} day, 4^{th} day, 28^{th} day, 180 days, 180 days, 1 year and 1 year after injection, respectively).

[0016] FIG. **9** is a photograph of a slow release of the fluorescently labeled macromolecules from the implant which includes crystallized dextran microparticles into mouse muscle tissue on the 14^{th} day after intermuscular injection.

[0017] FIG. **10** is a photograph of an expression of the reporter gene in a mouse's muscle tissue following the plasmid DNA release from the implant.

[0018] FIG. **11** is a photograph of an emulsion of aqueous solution of PEG in aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in FIG. **1**.

[0019] FIG. **12** is a photograph of an emulsion of aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in FIG. **1** in aqueous solution of PEG.

[0020] FIG. **13** is a photograph of an intramuscular injection of emulsion of aqueous solution of PEG in aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in FIG. **1**.

[0021] FIG. **14** is a photograph of a subcutaneous injection of emulsion of aqueous solution of PEG in aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in FIG. **1**.

[0022] FIGS. **15**A and **15**C schematically illustrate partition behavior of different types of particles and phases in an aqueous two phase system.

[0023] FIG. **15**B is a photograph of a cross section of an implant structure based on the two phase system.

[0024] FIGS. **16**A, **16**B, **16**C and **16**D are photographs of partition of HeLa cells and crystallized dextran microparticles in a two phase system.

[0025] FIGS. **17** and **18** schematically illustrate cell therapy methods according to embodiments of the present invention.

[0026] FIGS. **19**, **20** and **21** schematically illustrate therapeutic agent delivery methods according to embodiments of the present invention.

[0027] FIGS. **22**A and **22**B are graphs of relative normalized of blood glucose concentrations for various insulin containing composition versus time.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

A. Microparticle Formation

[0028] The present inventor has experimentally found that crystallized dextran microparticles with an average diameter ranging from 0.5 to 3.5 microns were spontaneously formed in concentrated aqueous solutions of dextrans (40-65% W/W) with molecular weights ranging from 1.0 to 200.0 kDa, at temperature ranging from 20-90° C. If it is desired to form the microparticles at room temperature, then 2 to 18 kDa dextran solutions may be used. Of course, the microparticles may also be formed from 2 to 18 kDa solutions at temperatures above room temperature, if desired. The microparticles may be spontaneously formed from higher molecular weight dextran solutions, such as 20 to 75 kDa solutions, at higher temperatures above room temperature, such as about 40 to about 70° C. The microparticles may have any suitable shape such as a regular or an irregular shape, but are preferably spherical in shape, and are preferably 10 microns in diameter or less, such as 0.5 to 5 microns.

[0029] Transmission Electron Microscopy revealed the microporous structure of the crystallized dextran microparticles (see FIGS. **2**A, **2**B). Preferably, the microparticle porosity is at least 10 percent by volume, such as about 10 to about 50 percent, more preferably about 20 to about 40 percent. Thus, the structure comprises microporous microparticles with areas of macroporosity located between the particles.

[0030] Spray drying of aqueous suspensions of the crystallized dextran microparticles has shown the possibility to produce substantially spherical aggregates of crystallized dextran microparticles with a diameter ranging from 10.0 to 150.0 microns (see FIG. **3**).

[0031] A non limiting example of a method of forming the dextran microparticles is as follows. 50.0 g of dextran T40 (40 kDa molecular weight) from Amersham Biosciences is added to 50.0 g of sterile distilled water in a 500 ml lab beaker to obtain 50% w/w solution under laminar flow. The mixture is stirred at 60° C. (water bath) on a magnetic stirrer at 50 rpm until the dextran is completely dissolved and a clear solution is obtained. The solution can be vacuumed to remove all air inclusions. The clear solution is placed in lab oven at 60° C. under a Tyvek® lid. 3.5 hours later, a turbid viscous suspension is developed as a result of formation of crystallized dextran microparticles.

[0032] To eliminate non-crystallized dextran, the microparticles are washed by centrifugation, for example 3,000 g, 30 min, with 3×250 ml of distilled sterile water, or by filtration of diluted suspension of micro particles, for example one part microparticles and 10 parts water (3×250 ml of distilled sterile water through sterilization filter). The centrifugation/washing is done under laminar flow. The microparticles are placed in 500 ml lab beaker under a Tyvek® lid and dried at 60° C. in lab oven for 8 hours to reach a moisture level of about 5%. The resulting dry powder consists of particles with a mean diameter of about 2 microns.

B. Implants Based on Crystallized Dextran Microparticles

[0033] Concentrated suspensions of the crystallized dextran microparticles and aggregates thereof were tried as implants in experiments with mice to test their biocompatibility following injection into the animal's body.

[0034] FIGS. **4** and **5** show the implant in tissue following subcutaneous (FIG. **4**) and intra-muscular (FIG. **5**) injections in experimental animals (mice). No inflammation reactions were detected in the animal's tissue during 180 days.

[0035] FIGS. **6**A, **6**B and **6**C show the implant in muscle tissue of a mouse 1, 4 and 28 days, respectively, after the injection. FIGS. **7**A and **7**B both show the implant in muscle tissue of a mouse 180 days after the injection.

[0036] FIGS. **8**A, **8**B and **8**C show the implant on the 4th, 11th and 28th day, respectively, after the subcutaneous injection. FIGS. **8**D and **8**E both show the implant 180 days after the subcutaneous injection. FIGS. **8**F and **8**G both show the implant one year after the subcutaneous injection. As shown in FIG. **8**G, normal tissue rather than scar tissue forms at the implant site.

[0037] The slow release of macromolecules from implants has been demonstrated in experiments where macromolecules were dissolved in aqueous suspensions of crystallized dextran microparticles or their aggregates before injections.

[0038] FIGS. **9** and **10** show the implant containing fluorescently labeled macromolecules (FITC-dextran, MW 500 kDa) and slow release of the macromolecules from the implant into a mouse muscle tissue on the 14^{th} day after the intermuscular injection (FIG. **9**) and expression of the reporter gene in a mouse's muscle tissue following the plasmid DNA release from the implant (FIG. **10**). Thus, the crystallized dextran microparticles may be used for timed release of a label, such as a fluorescent label alone or in combination with a therapeutic agent.

C. Implants Based on Two-Phase Systems

[0039] Self assembled structures of implants based on crystallized dextran microparticles and their aggregates may be formed based on two phase systems.

[0040] Colloidal systems such as droplets of oil, liposomes, micro- and nano-particles can be dispersed in a suspension of crystallized dextran microparticles and injected to form an implant releasing therapeutic agent(s) following administration into the mammal body.

[0041] For example, in the case of oil, a special kind of implant structure can be formed where the oil core is sur-

rounded with a shell composed of crystallized dextran microparticles or aggregates thereof dispersed in water or aqueous solutions of organic polymers such as polysaccharides (e.g. dextrans). The structure described can be designated as a capsule. It should be noted that the shell may comprise a roughly spherical shaped shell which results when the capsule is surrounded by tissue. However, when the capsule is located near a barrier, such as a substrate, bone or intestine wall, the capsule may comprise a core located between one or more walls of microparticles on one side and the barrier on the other side. Furthermore, while oil is used as an illustrative example, the core may comprise other materials, such as other polymers, cells, etc.

[0042] To form the capsule structure, two-phase aqueous systems are applied. When aqueous solutions of different polymers are mixed above certain concentrations they frequently form immiscible-liquid two-phase solutions. Each of the phases usually consists of more than 90% water and can be buffered and made isotonic. If a cell or particle suspension is added to such a system, the cells or particles are frequently found to have partitioned unequally between phases. This preferential partition behavior can be used as a basis for separation procedures for differing cell populations or particles since partition in these systems is determined directly by cell or particle surface properties. Cells or particles which do not have identical surface properties exhibit sufficiently different partition behavior.

[0043] The competitive adsorption of the two polymer phase depends on the chemical nature of the polymers. A two-phase polymer method has been applied to separate or partition cells, proteins, nucleic acids and minerals ("Partitioning in Aqueous Two-Phase Systems", 1985, eds., H. Walter, D. Brooks, and D. Fisher, pubis. Academic Press).

[0044] The experiments with the distribution of crystallized dextran microparticles in phase systems derived from, for instance, dextran/polyethylene glycol (PEG) mixtures, revealed that the dextran microparticles prefer to be in the dextran phase, while another PEG phase can be dispersed in this dextran phase to form a W/W emulsion and vice versa in the case when the volume of the PEG phase is bigger than the volume of the dextran phase, as shown in FIGS. **11** and **12**.

[0045] FIG. **11** is a photograph of an emulsion of aqueous solution of PEG in aqueous solution of dextran containing crystallized dextran microparticles. In the structure of FIG. **11**, the volume of the PEG phase is less than the volume of the dextran phase. The dextran phase contains the dextran and the crystallized dextran microparticles. Thus, the PEG phase forms into one or more sphere shaped cores surrounded by dextran/dextran microparticle shells (i.e., a closed pore structure).

[0046] FIG. **12** is a photograph of an emulsion of aqueous solution of dextran containing crystallized dextran microparticles in aqueous solution of PEG, where the volume of the PEG phase is greater than the volume of the dextran phase. In this case, the dextran phase forms into one or more sphere shaped cores containing the dextran microparticles surrounded by a PEG phase (i.e., an open pore structure that is forming in vivo while PEG dissipates in tissue liquid). As can be seen in FIG. **12**, the smaller volume (droplet) dextran phase forms into a large spherical dextran/dextran microparticle core (bottom right of FIG. **12**) to which smaller spheres comprising dextran/dextran microparticles are joining and fuse with.

[0047] Thus, when the ratio of the volume of the first phase (such as the PEG phase and its inclusions, such as a therapeutic agent) to the volume of the second phase (such as the dextran phase and its inclusions, such as the dextran microparticles) is less than one, then the capsule forms by self assembly with a first phase core surrounded by a second phase shell. If the composition contains a therapeutic agent, such as insulin, which prefers to partition into the PEG phase, and the dextran microparticles which prefer to partition into the dextran phase, then the therapeutic agent selectively partitions into the PEG core while the microparticles selectively partition into and form the shell around the PEG core by self assembly.

[0048] The emulsion can be prepared by the mixing of separately prepared dextran and PEG phases and both can be suspensions of different types of particles that prefer to be in the PEG phase or in the dextran phase respectively. The principle is that the partition of molecules (such as macromolecules, DNA, plasmids, etc.) or molecular aggregates (such as microparticles, cells, liposomes, proteins, etc.) into different polymer phases depends on their surface structure and interfacial energy of the particles in the polymer solutions.

[0049] Injection of aqueous two phase systems containing crystallized dextran microparticles into tissues of experimental animals revealed the formation of implants with the capsule structure as shown in FIGS. **13** and **14**. The volume of the dextran phase is greater than the volume of the PEG phase in the two-phase system. Both FIGS. **13** and **14** show that a capsule with a PEG core and a dextran/dextran microparticle shell forms by self assembly in vivo (i.e., after injection into mammal tissue). The shell comprises macroporous regions between adjacent microparticles as well as microporous regions in the microparticles themselves.

[0050] A non limiting example of a method of forming a capsule structure from a two phase system is as follows. 10 g of dextran T40 (40 kDa molecular weight) and 2 g of PEG are dissolved in 88 ml of (Actrapid®) insulin solution containing 1,000 UI to which 25 g of crystallized dextran microparticles are added. These steps are performed under laminar flow conditions. The mixture is stirred on a magnetic stirrer at 100 rpm at room temperature for 30 minutes to form a homogeneous mixture (i.e., a suspension). 1.0 g of the suspension contains 8 UI of insulin.

[0051] It should be noted that the dextran microparticles may be prepared from a different molecular weight dextran solution than the dextran solution which is provided in the two phase system. Thus, the crystallized dextran microparticles may be formed in a lower molecular weight dextran solution, such as a 2 to 20 kDa solution, than the dextran solution which is provided into the two phase system, which may be a 40 to 500 kDa dextran solution, such as a 40 to 75 kDa solution. This is advantageous because the higher molecular weight dextran solutions, such as 40 and 70 kDa solutions, have received wider regulatory approval and can be used to form a shell of a capsule at lower concentrations. The lower molecular weight solutions may be used to decrease the crystallization time without the lower molecular weight dextran solution actually being provided in vivo. Furthermore, lower molecular weight microparticles may dissolve easier in vivo.

[0052] The capsule structure formed from a two phase system is advantageous because it allows for a more even and prolonged release of the therapeutic agent from the core than

from a composition comprising a single phase containing the microparticles. Furthermore, it is believed that by using the capsule structure, a lower amount of microparticles may be needed to achieve the same or better timed release of a therapeutic agent than if a single phase system is used. Furthermore, by controlling the amount of microparticles in the two phase system, it is believed that the thickness of the microparticle shell may be controlled. A thicker shell results from a larger amount of microparticles in the two phase system. Thus, the amount, duration and/or timing of the release of the therapeutic agent from the capsule core may be controlled by controlling the thickness of the shell. Therefore, the release profile of the therapeutic agent may be customized for each patient or groups of patients.

[0053] It should be noted that while PEG and dextran are used as examples of the materials of the two phases, any other suitable materials which show the following partition behavior may be used instead. FIG. **15**A schematically illustrates partition behavior of different types of particles in an aqueous two phase system. For example, three types of molecules or molecular aggregates, which are preferably particles **10**, **12** and **14**, and two phases **16** and **18** are shown in FIG. **15**A. However, there may be two, or more than three types of particles. The particles may be microparticles such as microspheres or nanospheres prepared from organic and/or inorganic materials, liposomes, living cells, viruses and macromolecules. The first type particles **10** preferentially segregate into the first phase **16**. The second type particles **12** preferentially segregate to the boundary of the first **16** and second **18** phases. The third type particles **14** preferentially segregate into the second phase **18**. Thus, by analogy to the previous non-limiting example, the first particles **10** may comprise a therapeutic agent, the second **12** and/or the third **14** particles may comprise crystallized dextran microparticles, the first phase **16** may comprise a PEG phase and the second phase **18** may comprise a dextran phase.

[0054] If a smaller amount of the first phase **16** is provided into a larger amount of the second phase **18**, as shown in area **20** of FIG. **15**A, then a capsule type structure forms comprising discreet spheres of the first phase **16** containing a concentration of the first type particles **10**, located in a second phase **18**. The second type particles **12** may be located at the interface of the phases **16**, **18** and act as a shell of the capsule. Particles **14** are dispersed in the second phase **18** and/or form a shell of the capsule.

[0055] In contrast, if a smaller amount of the second phase **18** is provided into a larger amount of the first phase **16**, as shown in area **22** of FIG. **15**A, then a capsule type structure forms comprising discreet spheres of the second phase **18** containing a concentration of the third type particles **14**, located in a first phase **16**. The second type particles **12** may be located at the interface of the phases **16**, **18** and act as a shell of the capsule. Particles **10** are dispersed in the first phase **16** and/or form a shell of the capsule. The two phase systems **20** and **22** may be used as an implant, such as by being injected, surgically implanted or orally delivered into a mammal, such as an animal or human. Thus, the capsule forms a structured, three dimensional implant, with the core acting as a reservoir or depot for controlled release of the therapeutic agent through the shell. In contrast, an implant with an even distribution of microparticles is an unstructured implant. It should be noted that the structure formed for orally delivered two phase systems may be generally described as a structured suspension comprising a dispersed PEG phase and a continuous dextran phase.

[0056] Furthermore, particles (i.e., molecular aggregates) **10**, **12** and **14** may be substituted by a liquid material (e.g. oils) or macromolecules which selectively partition into one of the phases. For example, a therapeutic agent, such as insulin, may be partitioned in PEG phase of the PEG/dextran two phase system. Since insulin selectively partitions into the PEG phase, the PEG phase forms an insulin containing core of a capsule structure. It should be noted that while certain particles and therapeutic agents selectively partition, the term "selectively partitioned" does not necessarily mean that 100 percent of the particles or therapeutic agent partition into one of the phases. However, a majority of the selectively partitioned specie, preferably 80% of the partitioned specie, partitions into one of the phases. For example, while a majority of insulin partitions into the PEG phase, a portion of insulin may remain in the dextran phase.

[0057] FIG. **15**B illustrates a scanning electron microscope image of a cross section of an implant structure based on the two phase system schematically illustrated in FIG. **15**A. A two phase aqueous composition comprising a first dextran phase, a second PEG phase and crystallized dextran microparticles was injected into sepharose gel. This gel's composition mimics mammal tissue by stopping crystallized dextran microparticles diffusion from the injection side. The image in FIG. **15**B illustrates the formation of a core-shell implant structure. The core comprises regions **30** and **32** surrounded by a shell **34**. Region **30** is a void that is filled with a PEG phase region prior to cutting the gel for cross sectional SEM imaging. The PEG phase region drips out of the gel when the gel is cut during cross sectioning. Region **32** is an outer portion of the core comprising PEG droplets located in the crystallized dextran microparticles. Region **34** is the shell comprising the crystallized dextran microparticles which surrounds and holds in place the PEG containing core.

[0058] Without wishing to be bound by a particular theory, the present inventor believes that the core-shell structure shown in FIG. **15**B forms by self assembly as shown schematically in FIG. **15**C. While the first **16** and second **18** phases, such as aqueous solutions of different, incompatible polymers, are in a suitable storage container **19**, such as in a glass beaker or vial, one phase **16** rises above the other phase **18**. When the two phase composition is injected into a material which restricts free flow of the phases **16** and **18**, such as mammal tissue or a substrate material, such as a gel which mimics the tissue, the composition self assembles into the core-shell structure. First, the phase that is present in the smaller volume forms into approximate spherical shapes, as shown in the middle portion of FIG. **15**C. Then the spherical shapes join to form approximately spherical cores of one phase surrounded by shells of the other phase, as shown in the bottom of FIG. **15**C. While a two phase system example of a multiphase system has been illustrated, the multiphase system may have more than two phases if desired.

D. Cell Therapy

[0059] Experiments conducted with mammalian cells have shown possibilities to use the technique just described to deliver into a body the cells adsorbed by the surface of crystallized dextran microparticles aggregates (see FIGS. **3** and

12) or absorbed by the same surface being inside of macroporous structures (see FIG. **11**) or capsules (see FIGS. **13** and **14**).

[0060] In general, a self organizing material for therapies based on spontaneous organization in colloidal systems may contain crystallized dextran microparticles or other particles, such as PLA, PLGA, PMMA, alginate, cells, etc, for implants. For example, FIGS. **16**A-**16**D illustrate a partition of HeLa cells and crystallized dextran microparticles in a dextran-PEG two phase system. In FIG. **16**A, a two phase system is illustrated. The core comprising the PEG phase containing the cells is shown in the upper part of the photograph and the dextran/dextran microparticle ("CDM") shell is shown in the lower part. Droplets of the PEG phase segregating out of the shell into the core are shown at the boundary of the core and shell regions. FIG. **16**B shows partition of dextran microparticles in a PEG/dextran two phase system. The PEG phase **16** is on top and the dextran phase **18** containing the dextran microparticles is on the bottom. FIGS. **16**C and **16**D show HeLa cells on a dextran phase containing crystallized dextran microparticles surface.

[0061] The implants can be "reservoir" type implants and may be used for cell replacement therapy. "Reservoir" type implants can improve pharmacokinetics (no "burst effects") and ensure sustained release of biologically active substances. The "core" of the "reservoir" type implant may contain any type of active substance(s) in addition to the structure of the implant being "loaded" with cells. The core can contain therapeutic peptides and proteins, nucleic acids, vaccines, viruses and cells.

[0062] FIG. **17** schematically illustrates an example of cell replacement therapy based on self organizing material for therapies principle. For example, closed pore implants based on crystallized dextran microparticles may be used for type I diabetes therapy, cancer therapy, Parkinson's therapy and other suitable therapies based on the use of therapeutic proteins and peptides. In FIG. **17**, the implant **40** contains a crystallized dextran microparticle **12**, **14** capsule or shell encapsulating transplanted cells **42**. Any suitable cells may be used, such as insulin producing beta-cells, K cells, pancreatic cells or stem cells. As shown in FIG. **17**, the crystallized dextran microparticles capsule is porous to blood glucose **44** (as well as to oxygen, nutrients and other stimuli), but is impermeable to immune system cells which are too large to enter the capsule to attack the insulin producing cells. The blood glucose **44** permeates through the crystallized dextran microparticles capsule and stimulates production of insulin **46** from the insulin producing cells. The insulin then diffuses through the crystallized dextran microparticles capsule into the blood **48**.

[0063] Thus, crystallized dextran microparticles encapsulated insulin producing cells may be implanted into a mammal, such as a human, to treat diabetes. The crystallized dextran microparticles capsule may be formed in vivo and does not need a surgical operation. Such technique is known in the art as "Injectable Tissue Engineering". Furthermore, the crystallized dextran microparticles capsule does not require the use of toxic cross linking chemicals. The implants preferably may be used to provide a long acting insulin preparation to suppress hepactic glucose production and maintain near normoglycemia in the fasting state with a time-action profile without a peak.

[0064] It should be noted that the cell therapy may be used to treat other diseases. For example, crystallized dextran microparticles encapsulated dopamine producing cells may be used to treat Parkinson's disease, as illustrated schematically in FIG. **18**. Furthermore, as shown in FIG. **18**, the structure of the crystallized dextran microparticles capsule may be reversed, such that the cells **42** form the shell which encapsulate the microparticles **12**, **14** in the mammal tissue **50**. For example, patient's own cells or stem cells may be located outside the microparticles. In contrast, cells from a source other than the patient, such as from a donor, may be located inside the microparticle shell as shown in FIG. **17** to protect the cells from the patient's autoimmune reaction. This opens possibilities for in vivo tissue engineering for the treatment of conditions, such as diabetes and others.

E. Bone Graft Substitutes

[0065] Bone graft procedures involve the use of either autograft tissue, in which tissue is harvested from the patient, or allograft tissue, which is taken from donors or cadavers. Two basic criteria are used to judge a successful graft—osteoconduction and osteoinduction.

[0066] Harvesting autograft tissue requires surgery at the donor site that can result in its own complications, such as inflammation, infection, and chronic pain. Quantities of bone tissue that can be harvested are also limited, creating a supply problem as well.

[0067] Allografts circumvent some of the shortcomings of autografts by eliminating donor site morbidity and issues of limited supply. However, allografts present risks as well. Although many methods can reduce the risk of disease transmission, the treatments used to sterilize the tissue remove proteins and factors, reducing or eliminating the osteoinductivity of the tissue.

[0068] Furthermore, several alternatives have been developed to autografts and allografts. Many of these alternatives use a variety of materials, including natural and synthetic polymers, ceramics, and composites. Other alternative methods incorporate factor- and cell-based strategies that are used either alone or in combination with other materials.

[0069] Many bone graft substitutes are natural or synthetic and may be used alone or in combination with recombinant growth factors such as transforming growth factor (TGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and bone morphogenetic protein (BMP). Cell-based bone graft substitutes use cells to generate new tissue alone or are seeded onto a support matrix (e.g., mesenchymal stem cells).

[0070] With polymer-based bone graft substitutes, both degradable and nondegradable polymers are used alone or in combination with other materials, such as the Cortoss® open porosity poly-lactic acid polymer (OPLA) from Orthovita, Inc. Degradable synthetic polymers, like natural polymers, are resorbed by the body. The benefit of having the implant resorbed by the body is that the body is able to completely heal itself with no foreign bodies remaining. To this end, companies have used degradable polymers such as polylactic acid and poly-lactic-co-glycolic acid (PLGA) as stand-alone devices and as extenders to autografts and allografts. For example, Bone Tec, Inc, has developed a porous PLGA foam matrix by using a particulate leaching process to induce porosity. OsteoBiologics, Inc, has Immix Extenders, a particulate PLGA product used as a graft extender.

[0071] In one preferred aspect of this embodiment, the porous crystallized dextran microparticles described in the previous embodiment are used as bone graft substitutes, in

combination with a therapeutic agent which provides bone formation in special sites of said body. Any suitable therapeutic agent for bone formation may be used, such as a peptide, stem cell or protein, including but not limited to the above mentioned mesenchymal stem cells, TGF, PDGF, FGF and BMP. The therapeutic agent may be located in the pores of the porous microparticles. For example, the therapeutic agent may be provided into the pores of the crystallized dextran microparticles after crystallization.

[0072] In another preferred aspect of this embodiment, a method for in vivo implant formation includes preparing a suspension of microparticles in a first liquid phase, preparing a suspension of microparticles in a second liquid phase immiscible with the first liquid phase, preparing an emulsion where the first liquid phase is a continuous phase and the second liquid phase is a dispersed phase, and injecting of the emulsion into a mammal body. Preferably, the emulsion is used as a bone graft substitute.

[0073] Preferably, the microparticles in the first and second liquid phases are different. For example, the microparticles in the first phase may be non-polymer microparticles, such as porous ceramic microparticles, while the microparticles in the second phase may be polymer microparticles, such as the above mentioned porous crystallized dextran microparticles. If desired, the second liquid phase may contain the therapeutic agent providing bone formation in special sites of the body. The therapeutic agent may be located in the pores of the porous microparticles.

F. Oral Insulin Delivery Vehicle

[0074] In another preferred embodiment of the present invention, the present inventor discovered that a composition of porous (i.e., microporous) microparticles may be used as a vehicle for oral delivery of proteins, such as insulin. The porous microparticles may be any suitable porous microparticles which enable oral administration of insulin with a significant reduction in blood glucose; such as a 30% reduction within 60 minutes of oral administration. Preferably, the microparticles are bioadhesive particles, such as particles which adhere at least temporarily to mammal intestine walls, to allow insulin deliver through the intestine wall. Most preferably, the porous microparticles comprise the crystallized dextran microparticles described above.

[0075] In one preferred embodiment of the present invention shown in FIG. **19**, the present inventor has discovered that an aqueous suspension of crystallized dextran microparticles **12**, **14** and insulin **46** orally administered to mammals **53**, such as rabbits, was about equally as effective in reducing blood glucose levels as an intramuscular injection of insulin alone. FIG. **19** schematically illustrates the insulin **46** permeating through mammal **53** intestine **52** walls from the orally administered composition **54** comprising the microparticles. Since rabbits are a common model for humans in drug testing, the present inventor believes that a liquid or solid composition **54** comprising crystallized dextran microparticles and insulin, such as an aqueous suspension, a solution, a tablet or a capsule, would also be effective in reducing blood glucose levels in human beings when orally administered.

[0076] The following examples illustrate oral insulin delivery using crystallized dextran microparticles. The study involved Chinchilla rabbits (2.3±0.2 kg) and the observation of their response to orally administered aqueous suspensions consisting of crystallized dextran microparticles prepared according to the method described herein and human recombinant insulin.

[0077] 3.0 g of Dextran T20 (Pharmacia, Uppsala, Sweden) was dissolved in 2.0 g of water and placed in box at temperature 60° C. Three hours later, crystallized dextran microparticles were washed by centrifugation at 3,000 g with 3×5.0 ml of water. Then the crystallized dextran microparticles were suspended in 2.0 ml of water and allowed to dry at room temperature. The resulting dry powder was used to prepare an insulin containing suspension for the oral insulin delivery experiment. Insulin containing suspensions were prepared by the mixing of 250 mg of the microparticles; 0.3 ml (12 UI) or 0.6 ml (24 UI) of insulin (NovoNordisk Actrapid HM Penfill, 40 UI/ml); and distilled water to reach a volume of 2.0 ml.

[0078] Samples of the suspension (2.0 ml) were introduced into the rabbits' throats with a catheter followed by the introduction of 10.0 ml of drinking water. Animals were not fed for 3 hours before the suspension's introduction. Samples of blood were taken from the rabbit's ear vein and analyzed for glucose concentrations. Blood glucose was measured using the glucose oxidase method on a "One-touch System Glucose Analyzer" (Lifescan Johnson & Johnson, Milpitas, Calif., USA) after proper calibration.

[0079] Examples 1 to 8 are comparative examples involving eight rabbits. Examples 9 to 14 are examples according to the present invention involving five rabbits.

[0080] In comparative examples 1 and 2 (control experiment #1 summarized in Table I) an aqueous solution of human recombinant insulin was introduced intra muscularly into two rabbits at a dose of 12 UI per animal. In comparative examples 3 and 4 (control experiment #2 summarized in Table II), the rabbits remained intact (i.e., no insulin or other injection was provided to the two rabbits). In comparative examples 5 and 6 (control experiment #3 summarized in Table III), a suspension of the crystallized dextran microparticles without insulin was provided orally to two rabbits. In comparative examples 7 and 8 (control experiment #4 summarized in Table IV), a suspension of the commercially obtained Sephadex® G-150 microparticles with insulin (24 UI) was provided orally to two rabbits. According to the Amersham Biosciences website, Sephadex® G-150 microparticles are beaded gel microparticles having a diameter of 20 to 150 microns, prepared by cross linking dextran with epichlorohydrin. In examples 9-14 according to a preferred embodiment of the present invention (summarized in Table V), a suspension of crystallized dextran microparticles with insulin (24 UI) was provided orally to five rabbits. The results are summarized in Tables I-V below.

TABLE I

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#1	12 UI i.m.	91	68	58	49	51
#2	12 UI i.m.	87	65	64	57	58

TABLE II

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#3	0.0	98	87	87	89	86
#4	0.0	88	90	91	94	87

TABLE III

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#5	0.0	92	99	94	95	92
#6	0.0	90	93	93	93	92

TABLE IV

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#7	24 UI per os	85	82	86	81	83
#8	24 UI per os	84	75	86	76	77

TABLE V

Rabbit/ Experiment #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#9	24 UI per os	94	68	59	58	57
#10	24 UI per os	93	64	52	54	52
#11	24 UI per os	78	52	51	49	48
#12	24 UI per os	92	64	52	53	47
#13	24 UI per os	89	53	48	38	49
#14	24 UI per os	97	60	38	54	52

[0081] The data in Tables I-V show that average reduction of sugar (i.e., glucose) in the blood of mammals is comparable when 12 UI of insulin is administered by intramuscular injection (examples 1-2) and 24 UI of insulin is administered per os (i.e., orally) with crystallized dextran microparticles (examples 9-14). The maximum glucose reduction was about 35 to about 60 percent at 60 min after oral administration. The concentration profile of glucose is practically the same in both the injection and oral delivery modes. It should be noted that other amounts of insulin may be administered. For example, 30 UI of insulin may be administered. In general, oral administration of two to three times the insulin compared to the amount of injected insulin produces a similar drop in blood sugar for up to three hours.

[0082] It is a well known fact that insulin by itself is degraded by intestinal enzymes and is not absorbed intact across the gastrointestinal mucosa (Amidon G L, Lee H J, Absorption of peptide and peptidomimetic drugs, Ann. Rev. Pharmacol. Toxicol. 1994; 34: 321-41). However, examples 9-14 show that crystallized dextran microparticles can be used as a vehicle for oral delivery of proteins, such as insulin because the hypoglycemia effect received was significant. Without wishing to be bound by a particular theory or mode of action, the present inventor believes that the use of the porous, crystallized dextran microparticles as an insulin delivery matrix in an aqueous suspension protected the insulin from significant degradation by intestinal enzymes and allowed the insulin to be absorbed intact across the gastrointestinal mucosa. The insulin may be located in micropores in the microporous microparticles and/or in macropores between the microparticles. In contrast, the use of cross-linked Sephadex G-150 dextran microparticles with insulin (Table IV, examples 7-8) did not produce an appreciable reduction in blood glucose concentration.

[0083] As provided in examples 9-14, the blood glucose concentration in the mammal is lowered by at least 5 percent, preferably at least 30 percent, 60 minutes after administering the composition containing the crystallized dextran microparticles and insulin to the mammal (i.e., the blood glucose value in the mammal at 60 minutes after administration of the suspension is at least 5 percent, preferably at least 30 percent lower than that measured right before administration of the suspension). Preferably, the blood glucose concentration in the mammal is lowered by at least 5 percent, such as at least 30 percent, preferably by about 35 to about 40 percent 30 minutes after administering the composition to the mammal. Preferably, the blood glucose concentration in the mammal is lowered by about 35 to about 60 percent, for example 35 to 45 percent 60 minutes after administering the suspension to the mammal. More preferably, the blood glucose concentration in the mammal is lowered during the entire period ranging from 30 to 240 minutes, such as 30 to 120 minutes, after administering the composition to the mammal compared to the blood glucose concentration right before administration. For example, the blood glucose concentration in the mammal is preferably lowered by at least 10 percent, preferably at least 30 percent, more preferably by at least 35 percent, such as by 35 to 45 percent during a period ranging from 30 to 240 minutes, preferably 30 to 120 minutes after administering the composition to the mammal.

[0084] Thus, a preferred embodiment of the present invention provides a method of lowering blood glucose in a mammal by orally administering a therapeutically effective amount of a composition comprised of crystallized dextran microparticles and insulin. A "therapeutically effective" amount of the compositions can be determined by prevention or amelioration of adverse conditions or symptoms of diseases, injuries or disorders being treated. Preferably, the composition comprises an aqueous suspension of crystallized dextran microparticles having an average diameter of about 0.5 to about 5 microns and insulin. Furthermore, the microparticles are preferably porous microparticles which are crystallized prior to adding the insulin to the suspension, such that the insulin is located in contact with a surface of the microparticles and/or in pores of the microparticles.

[0085] The crystallized microparticles preferably are comprised of dextran molecules (i.e., polymer molecules) that are held together by a plurality of hydrogen bonds, Van Der Waals forces and/or ionic bonds and having substantially no covalent bonds between the dextran molecules. Thus, the molecules in the microparticles are preferably not intentionally cross-linked (i.e., a cross linking step is not carried out) and the microparticles contain no covalent bonds between molecules or less than 10% covalent bonds between molecules.

[0086] While a one phase composition **54** comprising insulin and microparticles is illustrated in FIG. **19**, a two phase

composition, described above and illustrated in FIGS. **13**, **14**, **15**A, **15**B, **15**C and **16**B may also be used. For example, a two phase composition comprising a dextran phase, a PEG phase, crystallized dextran microparticles and insulin may be used. In vivo, the composition has a self assembled capsule structure comprising a crystallized dextran microparticle containing wall or shell and a PEG and insulin containing core.

[0087] Preferably, the mammal receiving the oral administration of insulin comprises a human in need of lowering blood glucose, such as a human suffering from diabetes. Thus, the preferred embodiment of the present invention provides a method of treating diabetes in a human in need of the treatment by orally administering the suspension of insulin and crystallized dextran microparticles described above.

[0088] Any therapeutically effective amount of insulin may be administered to the mammal. The amount of insulin may vary based on the type of mammal (i.e., human or rabbit), the weight of the mammal, the composition of the suspension, the amount of desired reduction of blood glucose and other factors. One non-limiting example of insulin content in the suspension is about 10 UI to about 2,500 UI of human recombinant insulin per one gram of suspension, such as about 12 UI to about 30 UI, such as 24 UI of human recombinant insulin. However, this amount may vary as desired.

[0089] The present invention should not be considered limited to the preferred embodiments described above. Other matrix material may be used for oral insulin delivery, such as organic or inorganic microporous particles. Preferably, the particles are microparticles which enhance insulin penetration through gastrointestinal mucosa and/or which stabilize the composition. Furthermore, while the suspension preferably contains only water solvent; a matrix; and an insulin solution or suspension; the delivery system may also contain additional materials. For example, the composition may contain a second phase such as the PEG phase of a two phase system. Thus, another preferred aspect of the present invention includes a method of lowering blood glucose in a mammal comprising of orally administering a composition comprising a therapeutically effective amount of insulin and a matrix material to the mammal to lower blood glucose of the mammal by at least 30 percent 60 minutes after administering the suspension to the mammal. In another preferred aspect of the present invention, a method of administering a suspension to a mammal comprised of orally administering an aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin to the mammal.

[0090] As noted above, the crystallized dextran microparticles used as a matrix material for oral administration of insulin or other protein based drugs may be made by any suitable method (see FIG. **1**, for example). Preferably, the microparticles are made by the process of any of the preferred embodiments described herein. Preferably, but not necessarily, the microparticles are formed in an aqueous solution without using an organic solvent. Thus, in a preferred aspect of the present invention, a therapeutically effective amount of insulin and the crystallized dextran microparticles are combined in water after the microparticles have been crystallized to form an aqueous suspension of insulin and crystallized dextran microparticles. The microparticles may be added to the water before, at the same time and/or after adding the insulin to the water. Furthermore, the microparticles may be orally administered to a mammal in the solvent in which they were formed. Alternatively, they may be removed from the solvent in which they were formed and placed into water or other aqueous solutions for oral administration or dried and provided in solid form, such as tablet or capsule, for oral administration.

[0091] The aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin (or other suitable suspensions of insulin and a matrix material, such as a suspension of insulin and microporous microparticles) is preferably provided as a dosed pharmaceutical composition which is dosed for oral administration to a human. In one preferred aspect of the present invention, the composition is located in a vessel in an amount dosed for a single oral administration to a human. The vessel may comprise any container which may hold a suspension, such as a plastic or glass bottle, a tube, a dropper, a spray nozzle, a pouch and/or other suitable vessels. This vessel contains an amount of suspension sufficient for a single oral dose of the composition.

[0092] In another preferred aspect of the present invention, the composition is located in any suitable vessel in an amount suitable for multiple oral administration doses. The vessel contains an instruction for oral dosage administration to a human. The instruction may be printed on the vessel, such being as printed directly on the vessel or on an label attached to the vessel, or enclosed with the vessel, such being printed on a sheet of paper enclosed with the vessel in a cardboard box or in a pharmacy envelope. The instructions may describe the amount of the composition that should be taken with each dose, the frequency that the dose should be taken, how to measure the dose of the composition for oral administration and/or any other suitable oral drug instructions for an administering health care practitioner and/or a patient in need of the drug. Alternatively, the instructions may comprise directions for electronically or audibly accessing the dosing and administration instructions, such as a link to a website containing the instructions or a telephone number or recording where the instructions are provided audibly.

[0093] In another preferred aspect of the present invention, the aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin located in a vessel are provided in a pharmaceutical composition kit with instructions for oral administration of the composition to a human in need thereof. The kit may comprise instructions printed on the vessel or on a label attached to the vessel or a sheet of paper enclosed with the vessel, such as in a cardboard box or pharmacy envelope including a bottle (i.e., vessel) and the sheet of instructions.

[0094] It should be noted that the composition for oral administration may be in the form of an aqueous suspension, but other delivery forms may be used to lower the blood glucose in a mammal. For example, the porous crystallized dextran microparticles and the insulin may be orally administered in the form of a tablet or a capsule.

[0095] To orally administer the composition in solid form to a mammal, such as a human, the solution of crystallized dextran microparticles and insulin is first dried, such as freeze dried, to form a powder. The powder may then be compressed into a tablet, along with optional pharmaceutically acceptable excipients or placed into a pharmaceutically acceptable capsule.

[0096] In another preferred aspect of the present invention, the composition comprising crystallized dextran microparticles and a therapeutically effective amount of insulin may be administered to a mammal, such as a human by inhalation. In this case, the composition is placed into a vessel adapted for

administering a pharmaceutical composition to a mammal by inhalation, such as an inhaler which provides a metered dose of a composition when squeezed or pressed. Preferably, the composition is provided to a mammal through the mouth by spraying the composition in solution or suspension form from the inhaler. Preferably, the composition is delivered to the lungs of the mammal (i.e., pulmonary delivery).

G. Injectable Insulin Delivery Vehicle

[0097] The present inventor has discovered that a composition of crystallized dextran microparticles and insulin injected into mammals, such as rats and rabbits, unexpectedly extended the duration of efficacy of the insulin compared to injections of the same dose of the same insulin alone. FIG. **20** schematically illustrates the formation of an implant **40** in a mammal **53** by injection of a one phase composition comprising the microparticles **12**, **14** and insulin **46** using a syringe **56**. FIG. **21** schematically illustrates the formation of a structured implant **40** in a mammal **53** by injection of a two phase composition comprising a dextran phase **18** containing selectively partitioned crystallized dextran microparticles **12**, **14** and a PEG phase **16** containing selectively partitioned therapeutic agent **10** comprising insulin. The dextran phase **18** forms a shell around the PEG phase **16** core. Since rats and rabbits are a common model for humans in drug testing, the present inventor believes that the comprising crystallized dextran microparticles and insulin would also be effective in extending the duration of efficacy of the insulin when injected into human adults and children.

[0098] Examples 15-22 illustrate the advantage of using crystallized dextran microparticles as an injectable insulin delivery vehicle compared to injected insulin alone. The experiment involved mice and the observation was made of their response to a subcutaneously injected aqueous suspension consisting of crystallized dextran microparticles and human recombinant insulin (NovoNordisk Actrapid HM Penfill®, 40 UI/ml).

[0099] The suspension was prepared as follows. 5.0 g of Dextran T10 (Pharmacia, Uppsala, Sweden) was dissolved in 20.0 g of water. The solution was filtered through a 0.22 μm filter (Millipore, Bedford, Mass.) and freeze dried. 3.0 g of the resulting powder was dissolved in 3.0 g of sterile water and placed in box at temperature 60° C. 6 hours later, crystallized dextran microparticles were washed by centrifugation at 3,000 g with 3×5.0 ml of sterile water. Finally, the produced crystallized dextran microparticles suspension was mixed with aqueous insulin solution and used in the experiment with mice. Samples of the suspension were introduced into the mice's legs and samples of animal blood were taken from each mouse's tail and analyzed for glucose concentrations. Blood glucose was measured using the glucose oxidase method on a One-touch system glucose analyzer (Lifescan, Johnson & Johnson, Milpitas, Calif., USA) after proper calibration.

[0100] In comparative example 15, no insulin was injected into the mouse. In comparative examples 16, 17 and 21, insulin alone (0.5 UI) was injected into the three mice. In examples 18-20 and 22, insulin (0.5 UI) and a crystallized dextran microparticles implant was injected into the four mice. The results are summarized in Table VI.

TABLE VI

Ex #		0 min glucose mmol/l	15 min glucose mmol/l	30 min glucose mmol/l	45 min glucose mmol/l	120 min glucose mmol/l	210 min glucose mmol/l	270 min glucose mmol/l	390 min glucose mmol/l
15	Intact mouse	7.9	8.1	8.2	8.4	—	—	—	—
16	Insulin 0.5 UI	5.9	3.3	2.7	1.8	0.9	3.5	3.0	3.2
17	Insulin 0.5 UI	8.1	3.8	2.8	1.9	0.9	3.7	3.4	3.5
18	Insulin 0.5 UI with crystallized dextran microparticles	6.0	4.3	3.2	2.5	0.8	0.8	0.9	0.7
19	Insulin 0.5 UI with crystallized dextran microparticles	6.9	5.6	4.1	3.4	—	1.2	—	1.6
20	Insulin 0.5 UI with crystallized dextran microparticles	5.9	3.5	2.9	1.9	1.2	1.0	1.0	0.7

TABLE VI-continued

Ex #		0 min glucose mmol/l	15 min glucose mmol/l	30 min glucose mmol/l	45 min glucose mmol/l	120 min glucose mmol/l	210 min glucose mmol/l	270 min glucose mmol/l	390 min glucose mmol/l
21	Insulin 0.5 UI (average)	7	3.6	2.8	1.9	0.9	3.6	3.2	3.4
22	Insulin 0.5 UI with crystal-lized dextran micro-particles (average)	6.3	4.5	3.4	2.6	1.0	1.0	1.0	1.0

[0101] The average reduction of sugar in the blood (i.e., blood glucose) of animals is very different when 0.5 UI i.m. were applied with and without crystallized dextran microparticles. As shown in Table VI, the glucose level in the mice of comparative examples 16, 17 and 21 is about the same or lower than the glucose level in mice of examples 18-20 and 22 during the first 45 minutes after injection. The glucose level is about the same in mice of both comparative examples 16, 17 and 21 and examples 18-20 and 22 120 minutes after injection. However, the glucose level in the mice of comparative examples 16, 17 and 21 is about three times higher than the glucose level in mice of examples 18-20 and 22 from 210 to 390 minutes after injection. In fact, the blood glucose level in mice in examples 18-20 and 22 did not substantially increase (i.e., did not increase by more than 10%, remained the same or decreased) from 120 minutes to 390 minutes after injection. In contrast, the blood glucose level in mice in the comparative examples 16, 17 and 21 injected with the same amount of insulin did substantially increase from 120 to 390 minutes after injection. The crystallized dextran microparticles/insulin injection decreases blood glucose for a longer time than an injection of insulin of the same dose alone. Thus, the composition containing crystallized dextran microparticles and insulin may be dosed for injection.

[0102] The following experiments on rabbits also demonstrate how the crystallized dextran microparticles/insulin injection decreases blood glucose and maintains a basal level of blood insulin for a longer time than an injection of the same insulin of the same dose alone. A subcutaneously injected composition comprising Actrapid HM® short-acting insulin and crystallized dextran microparticles was unexpectedly found to extend the duration of efficacy of this short-acting insulin to exceed that of subcutaneously injected, long-acting insulin Monotard HM® alone.

[0103] The term duration of efficacy means decreasing blood glucose concentration and/or maintaining a basal level of blood insulin concentration to desired levels independent of external events that cause spikes in blood glucose, such as eating. Thus, the term duration of efficacy is a relative term comparing the efficacy of the insulin and microparticle composition to that of the same dose of the same insulin alone. In other words, the duration of efficacy is a duration of action or a duration of pharmacological effect, which may be measured in a patient in a fasting state to compare the efficacy of the insulin and microparticle composition to that of the same dose of the same insulin alone.

[0104] As shown in FIGS. **22**A and **22**B, the composition comprising the Actrapid HM® short-acting insulin and crystallized dextran microparticles prolonged the absorption of insulin and extended the hypoglycemic effect (i.e., the duration of efficacy of the insulin) to at least twenty four hours, such as about twenty eight to about thirty one hours, as compared to about two to about eight hours for Actrapid HM® insulin alone (FIG. **22**B) and about seventeen to about twenty-four hours for "Monotard HM®" insulin alone (FIG. **22**A). Both Actrapid HM® and Monotard HM® insulins are products of Novo Nordisk and the advertised duration of efficacy of these insulin compositions in humans obtained from company information are eight and twenty four hours, respectively.

[0105] In FIGS. **22**A and **22**B, the upper line illustrates the control line for intact rabbits to which no insulin was administered. The y-axis of FIGS. **22**A and **22**B is a relative normalized scale of blood glucose concentration for the same 8 UI dose of insulin. The data in the Figures was adjusted to be shown in one plot for each figure and shows blood glucose levels in blood of animals following insulin injections.

[0106] The data shown in FIGS. **22**A and **22**B was obtained as follows. Chinchilla rabbits (2.3±0.3 kg) were monitored for their response to injections of a formulation consisting of crystallized dextran microparticles and short-acting insulin Actrapid HM®. Samples of the formulation were subcutaneously injected into the rabbits. Long acting insulin Monotard HM® (40 UI/ml) and short acting insulin Actrapid HM® were subcutaneously injected into separate rabbits without the microparticles and used as controls. Samples of animal blood were taken from the rabbit's ear vein and analyzed for glucose concentration. Blood glucose concentration was measured with a glucose analyzer (One-Touch® Lifescan, Johnson & Johnson, Milpitas, Calif., USA) after proper calibration.

[0107] In comparative examples 23 and 24, two intact rabbits were not provided any insulin. In comparative examples 25 and 26 an aqueous solution of long-acting insulin Monotard HM® was introduced subcutaneously to two rabbits in a dose of 8 UI. In examples 27-29, a suspension of crystallized dextran microparticles with short-acting insulin Actrapid HM® was introduced subcutaneously to three rabbits in a dose of 8 UI. The results of the experiments are summarized in Table VII.

TABLE VII

Ex #	Insulin dose	0 hours glucose mmol/L	0.5 hours glucose mmol/L	1 hour glucose mmol/L	1.5 hours glucose mmol/L	2 hours glucose mmol/L	2.5 hours glucose mmol/L	16 hours glucose mmol/L	24 hours glucose mmol/L	31 hours glucose mmol/L
23	0.0	5.4	5.0	5.2	5.2	5.2	5.4	4.7	5.4	5.4
24	0.0	6.0	6.3	6.3	6.3	6.3	6.4	5.7	5.6	5.8
25	8 UI	5.4	5.8	3.8	3.2	2.4	2.6	3.9	5.6	N.A
26	8 UI	5.4	5.0	4.2	2.9	2.5	2.4	4.0	5.1	N.A
27	8 UI	5.8	3.7	1.9	1.9	1.9	2.8	4.1	4.3	4.1
28	8 UI	6.6	5.7	4.3	3.9	3.7	3.9	4.6	4.1	3.9
29	8 UI	6.2	5.1	3.6	3.2	3.1	2.9	4.2	4.4	4.7

[0108] The above examples 27-29 illustrate that the composition of crystallized dextran microparticles with short-acting insulin Actrapid HM® provides a prolonged effect that exceeds the effect of long acting insulin Monotard HM® and is believed to be comparable to the effect of long acting (once daily dosing) insulin glargine Lantus® from Aventis (see www.aventis-us.com/Pls/lantus_TXT.html). In addition, Lantus® insulin must not be diluted or mixed with any other insulin or solution. If Lantus® insulin is diluted or mixed, the pharmacokinetic/pharmacodynamic profile (e.g., onset of action, time to peak effect) of Lantus® and/or the mixed insulin may be altered in an unpredictable manner. In contrast, the composition of crystallized dextran microparticles with insulin is not so limited because any suitable insulin, such as human insulin, may be used. In the composition of crystallized dextran microparticles and insulin, the ratio of insulin and microparticles can be varied as desired. Furthermore, any suitable insulin may be used to custom fit an insulin therapy to an individual patient. Thus, Actrapid HM® was used in the composition as an illustrative example of a typical insulin and the composition is not limited to this brand of insulin.

[0109] As shown in examples 23 to 29, the composition containing the crystallized dextran microparticles and insulin is effective in maintaining a duration of efficacy of the insulin for at least 30% longer, such as at least 100% longer, preferably 100 to 400% longer than the same dose of the same insulin without the microparticles. The microparticle containing insulin composition is effective in maintaining a desired basal level of blood insulin and blood glucose concentration for at least 30% longer, such as 100% to 400% longer, than the same dose of the same insulin without the microparticles. Thus, the duration of efficacy of the microparticle containing composition is at least 24 hours, which allows it to be injected only once daily into the mammal, such as a human in need thereof.

[0110] The long lasting insulin crystallized dextran microparticle composition is safer than prior art long lasting insulin compositions because it can achieve the long lasting efficacy without using a higher dose of insulin as in the prior art compositions. For example, if a 8 UI dose of short acting insulin has been determined medically safe for a patient without a significant risk of overdose, then the composition comprising the same short acting insulin and the crystallized dextran microparticles can provide longer acting duration efficacy at the same 8 UI dose of short acting insulin without a significant risk of overdose, even if all the insulin is released into the patient at once. Furthermore, this composition provides a cost saving compared to the prior art compositions because it extends the efficacy without increasing the amount of insulin. Current prior art long-acting diabetes therapies are made with analogs of insulin, such as the Lantus® insulin from Aventis. In contrast, the crystallized dextran microparticle containing composition preferably contains human recombinant insulin whose safety profile is established. Thus, this composition reduces the risk of adverse reaction(s) and number of injections to diabetics, thereby enhancing the quality of life of the diabetics.

[0111] The injectable composition may comprise a single phase system comprising insulin and microparticles or a two phase system which forms a PEG and insulin core and a dextran and dextran microparticle shell for an even greater duration of efficacy. Furthermore, the composition comprises a flowable one phase or multiphase colloidal system (i.e., a suspension or an emulsion) which is relatively easy to inject into a mammal.

[0112] The following example illustrates the use of an injectable two phase composition comprising a dextran phase, a PEG phase, insulin and crystallized dextran microparticles. It is believed that when injected into a mammal, this composition forms a structured reservoir type implant having a three dimensional capsule structure. In the capsule structure, the microparticles selectively partition into the dextran phase and the insulin selectively partitions into the PEG phase. The dextran phase containing the microparticles forms a shell around a core comprising the PEG phase containing the insulin. This structured implant allows for controlled release from the core through the shell.

[0113] In comparative example 30, 0.5 UI of Actrapid HM® insulin (100 UI/ml) is subcutaneously injected into a mouse. In example 31, 0.4 g of crystallized dextran microparticles are dispersed in 0.6 ml of 20% (W/W) aqueous solution of dextran having a molecular weight of 70 kDa (Pharmacia, Sweden) to form a suspension. 10 mg of PEG having a molecular weight 6 kDa (Fluke) is dissolved in 0.1 ml of Actrapid HM® insulin (100 UI/ml) to form a solution. 0.05 ml of the PEG and insulin solution is mixed with 0.15 ml of the microparticle and dextran suspension to form a two phase composition or mixture. 0.02 ml of the two phase mixture containing 0.5 UI of insulin is injected subcutaneously into mouse. The results are shown in Table VIII.

TABLE VIII

Example #	0 min glucose mmol/L	15 min glucose mmol/L	30 min glucose mmol/L	45 min glucose mmol/L	60 min glucose mmol/L	120 min glucose mmol/L
30	7.8	3.7	2.3	1.7	2.9	6.7
31	7.9	5.9	4.3	4.1	4.3	4.0

[0114] As can be seen in Table VIII, the two phase composition duration of efficacy was longer than that of the insulin alone. Furthermore, the two phase composition decreased the blood glucose concentration more gradually than insulin alone. Without wishing to be bound by a particular theory, these effects are believed due to the controlled insulin release from the core of capsule structure.

[0115] Furthermore, the microparticle containing composition may be individually tailored for each patient by adjusting the amount of insulin and/or microparticles to allow the patient to inject the composition at the same time every day (i.e., once every 24 hours, once every 48 hours, etcetera). Thus, the duration of efficacy of the composition is adjustable for each patient. For a two phase system, the insulin release profile from the core of the capsule may be adjusted by controlling the amount of microparticles to control the shell thickness of the capsule.

[0116] While the inventor does not wish to be bound by any particular theory, it is believed that the long lasting effect of the same dose of insulin in mice and rabbits with crystallized dextran microparticles can be explained by the diffusion of the insulin molecules from the crystallized dextran microparticles based implant (i.e., a self controlled release of insulin). Since mice and rabbits are a common model for humans in drug testing, the data shown in the above tables VI to VIII suggests that the use of crystallized dextran microparticles based implants makes it possible to develop controlled release delivery systems with improved pharmacokinetic and dynamics characteristics and that better meet the needs of basal insulin patients, such as humans.

H. Materials

[0117] The term "insulin" shall be interpreted to encompass insulin analogs, natural extracted human insulin, recombinant produced human insulin, insulin extracted from bovine and/or porcine sources, recombinant produced porcine and bovine insulin and mixtures of any of these insulin products. The term is intended to encompass the polypeptide normally used in the treatment of diabetics in a substantially purified form but encompasses the use of the term in its commercially available pharmaceutical form, which includes additional excipients. The insulin is preferably recombinant produced and may be dehydrated (completely dried) or in solution.

[0118] The terms "insulin analog," "monomeric insulin" and the like are used interchangeably herein and are intended to encompass any form of "insulin" as defined above, wherein one or more of the amino acids within the polypeptide chain has been replaced with an alternative amino acid and/or wherein one or more of the amino acids has been deleted or wherein one or more additional amino acids has been added to the polypeptide chain or amino acid sequences, which act as insulin in decreasing blood glucose levels. In general, the term "insulin analogs" of the preferred embodiments of the present invention include "insulin lispro analogs," as disclosed in U.S. Pat. No. 5,547,929, incorporated hereinto by reference in its entirety; insulin analogs including LysPro insulin and humalog insulin, and other "super insulin analogs", wherein the ability of the insulin analog to affect serum glucose levels is substantially enhanced as compared with conventional insulin as well as hepatoselective insulin analogs which are more active in the liver than in adipose tissue. Preferred analogs are monomeric insulin analogs, which are insulin-like compounds used for the same general purpose as insulin, such as insulin lispro, i.e., compounds which are administered to reduce blood glucose levels.

[0119] The term "analog" refers to a molecule, which shares a common functional activity with the molecule to which it is deemed to be comparable and typically shares common structural features as well.

[0120] The term "recombinant" refers to any type of cloned therapeutic expressed in prokaryotic cells or a genetically engineered molecule, or combinatorial library of molecules which may be further processed into another state to form a second combinatorial library, especially molecules that contain protecting groups which enhance the physicochemical, pharmacological, and clinical safety of the therapeutic agent.

[0121] Furthermore, the therapeutic agents described herein are not limited to insulin. Any other suitable therapeutic agent may be used in conjunctions with the microparticles for oral delivery, inhalation delivery, injection delivery and surgical implantation delivery.

[0122] For example, a suitable therapeutic agent may comprise a peptide, polypeptide, or protein ranging from 0.5 K Dalton to 150 K Dalton in molecular size. In particular, the peptide, polypeptide, or protein therapeutic agents include diabetic aids; such as the above mentioned insulin and insulin analogs; amylin; glucagon; surfactants; immunomodulating peptides and proteins such as cytokines, chemokines, lymphokines; taxol; interleukins, such as, interleukin-1, interleukin-2, and interferons; erythropoetins; thrombolytics and heparins; anti-proteases, antitrypsins and amiloride; rhDNase; antibiotics and other anti-infectives; hormones and growth factors, such as parathyroid hormones, LH-RH and GnRH analogs; nucleic acids; DDAVP; calcitonins; cyclosporine; ribavirin; enzymes; heparins; hematopoietic factors; cyclosporins; vaccines; immunoglobulins; vasoactive peptides; antisense agents; oligonucleotide, and nucleotide analogs. Other suitable therapeutic agents include viruses, cells, genes and other agents having therapeutic properties, such as other suitable vaccines and molecular therapeutic agents.

[0123] The term "amylin" includes natural human amylin, bovine, porcine, rat, rabbit amylin, as well as synthetic, semisynthetic or recombinant amylin or amylin analogs including pramlintide and other amylin agonists.

[0124] The term "immunomodulating proteins" include cytokines, chemokines, lymphokines complement components, growth hormones, immune system accessory and adhesion molecules and their receptors of human or non-human animal specificity. Useful examples include GM-CSF, G-CSF, IL-2, IL-12, OX40, OX40L (gp34), lymphotactin, CD40, CD40L. Useful examples include interleukins, for example interleukins 1 to 15; interferons alpha, beta or gamma; tumor necrosis factor, granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), chemokines, such as neutrophil activating protein (NAP); macrophage chemoattractant and activating factor (MCAF), RANTES, macrophage inflammatory peptides MIP-1a and MIP-1b, complement components and their receptors, or an accessory molecule, such as B7.1, B7.2, ICAM-1, 2 or 3 and cytokine receptors. OX40 and OX40-ligand (gp34) are further useful examples of immunomodulatory proteins. Immunomodulatory proteins can for various purposes be of human or non-human animal specificity and can be represented, for present purposes, as the case may be and as may be convenient, by extracellular domains and other

fragments with the binding activity of the naturally occurring proteins, and muteins thereof, and their fusion proteins with other polypeptide sequences, e.g. with immunoglobulin heavy chain constant domains. Where nucleotide sequences encoding more than one immunomodulating protein are inserted, they can, for example, comprise more than one cytokine or a combination of cytokines and accessory/adhesion molecules.

[0125] The term "interferon" or "IFN" as used herein means the family of highly homologous species-specific proteins that inhibit viral replication and cellular proliferation and modulate immune response. Interferons are grouped into three classes based on their cellular origin and antigenicity, namely, alpha-interferon (leukocytes), beta-interferon (fibroblasts) and gamma-interferon (immunocompetent cells). Recombinant forms and analogs of each group have been developed and are commercially available. Subtypes in each group are based on antigenic/structural characteristics. At least 24 interferon alphas (grouped into subtypes A through H) having distinct amino acid sequences have been identified by isolating and sequencing DNA encoding these peptides. The terms "alpha-interferon", "alpha interferon", "interferon alpha", "human leukocyte interferon" and "IFN" are used interchangeably herein to describe members of this group. Human leukocyte interferon prepared in this manner contains a mixture of human leukocyte interferons having different amino acid sequences.

[0126] The term "erythropoietin" applies to synthetic, semi-synthetic, recombinant, natural, human, monkey, or other animal or microbiological isolated polypeptide products having part or all of the primary structural conformation (i.e., continuous sequence of amino acid residues) and one or more of the biological properties (e.g., immunological properties and in vivo and in vitro biological activity) of naturally-occurring erythropoietin, including allelic variants thereof. These polypeptides are also uniquely characterized by being the product of procaryotic or eucaryotic host expression (e.g., by bacterial, yeast and mammalian cells in culture) of exogenous DNA sequences obtained by genomic or cDNA cloning or by gene synthesis. Products of microbial expression in vertebrate (e.g., mammalian and avian) cells may be further characterized by freedom from association with human proteins or other contaminants which may be associated with erythropoietin in its natural mammalian cellular environment or in extracellular fluids such as plasma or urine. The products of typical yeast (e.g., *Saccaromyces cerevisiae*) or procaryote (e.g., *E. coli*) host cells are free of association with any mammalian proteins. Depending upon the host employed, polypeptides of the invention may be glycosylated with mammalian or other eucaryotic carbohydrates or may be nonglycosylated. Polypeptides may also include an initial methionine amino acid residue (at position −1). Novel glycoprotein products of the invention include those having a primary structural conformation sufficiently duplicative of that of a naturally-occurring (e.g., human) erythropoietin to allow possession of one or more of the biological properties thereof and having an average carbohydrate composition which differs from that of naturally-occurring (e.g., human) erythropoietin.

[0127] The terms "heparins" and "thrombolytics" include anti-clotting factors such as heparin, low molecular weight heparin, tissue plasminogen activator (TPA), urokinase (Abbokinase) and other factors used to control clots.

[0128] The terms "anti-proteases" and "protease-inhibitors" are used interchangeably and apply to synthetic, semi-synthetic, recombinant, naturally-occurring or non-naturally occurring, soluble or immobilized agents reactive with receptors, or act as antibodies, enzymes or nucleic acids. These include receptors which modulate a humoral immune response, receptors which modulate a cellular immune response (e.g., T-cell receptors) and receptors which modulate a neurological response (e.g., glutamate receptor, glycine receptor, gamma-amino butyric acid (GABA) receptor). These include the cytokine receptors (implicated in arthritis, septic shock, transplant rejection, autoimmune disease and inflammatory diseases), the major histocompatibility (MHC) Class I and II receptors associated with presenting antigen to cytotoxic T-cell receptors and/or T-helper cell receptors (implicated in autoimmune diseases) and the thrombin receptor (implicated in coagulation, cardiovascular disease). Also included are antibodies which recognize self-antigens, such as those antibodies implicated in autoimmune disorders and antibodies which recognize viral (e.g., HIV, herpes simplex virus) and/or microbial antigens.

[0129] The terms "hormones" and "growth factors" include hormone releasing hormones such as growth hormone, thyroid hormone, thyroid releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), leuteininzing hormone, leuteininzing hormone-releasing hormone (LHRH, including the superagonists and antagonists, such as leuprolide, deltirelix, gosorelin, nafarelin, danazol, etc.) sourced from natural, human, porcine, bovine, ovine, synthetic, semi-synthetic, or recombinant sources. These also include somatostatin analogs such as octreotide (Sandostatin). Other agents in this category of biotherapeutics include medicaments for uterine contraction (e.g., oxytocin), diuresis (e.g., vasopressin), neutropenia (e.g., GCSF), medicaments for respiratory disorders (e.g., superoxide dismutase), RDS (e.g., surfactants, optionally including apoproteins), and the like.

[0130] The term "enzymes" include recombinant deoxyribonuclease such as DNAse (Genentech) proteases (e.g., serine proteases such as trypsin and thrombin), polymerases (e.g., RNA polymerases, DNA polymerases), reverse transcriptases and kinases, enzymes implicated in arthritis, osteoporosis, inflammatory diseases, diabetes, allergies, organ transplant rejection, oncogene activation (e.g., dihydrofolate reductase), signal transduction, self-cycle regulation, transcription, DNA replication and repair.

[0131] The term "nucleic acids" includes any segment of DNA or RNA containing natural or non-naturally occurring nucleosides, or other proteinoid agents capable of specifically binding to other nucleic acids or oligonucleotides via complementary hydrogen-bonding and also are capable of binding to non-nucleic acid ligands.

[0132] The term "vaccines" refers to therapeutic compositions for stimulating humoral and cellular immune responses, either isolated, or through an antigen presenting cell, such as an activated dendritic cell, that is able to activate T-cells to produce a multivalent cellular immune response against a selected antigen. The potent antigen presenting cell is stimulated by exposing the cell in vitro to a polypeptide complex. The polypeptide complex may comprise a dendritic cell-binding protein and a polypeptide antigen, but preferably, the polypeptide antigen is either a tissue-specific tumor antigen or an oncogene gene product. However, it is appreciated that other antigens, such as viral antigens can be used in such combination to produce immunostimulatory responses. In

another preferred embodiment, the dendritic cell-binding protein that forms part of the immunostimulatory polypeptide complex is GM-CSF. In a further preferred embodiment, the polypeptide antigen that forms part of the complex is the tumor-specific antigen prostatic acid phosphatase. In still other preferred embodiments, the polypeptide antigen may be any one of the oncogene product peptide antigens. The polypeptide complex may also contain, between the dendritic cell-binding protein and the polypeptide antigen, a linker peptide. The polypeptide complex may comprise a dendritic cell-binding protein covalently linked to a polypeptide antigen, such polypeptide complex being preferably formed from a dendritic cell binding protein, preferably GM-CSF, and a polypeptide antigen. The polypeptide antigen is preferably a tissue-specific tumor antigen such as prostatic acid phosphatase (PAP), or an oncogene product, such as Her2, p21RAS, and p53; however, other embodiments, such as viral antigens, are also within the scope of the invention.

[0133] The term "immunoglobulins" encompasses polypeptide oligonucleotides involved in host defense mechanisms, such as coding and encoding by one or more gene vectors, conjugating various binding moieties of nucleic acids in host defense cells, or coupling expressed vectors to aid in the treatment of a human or animal subject. The medicaments included in this class of polypeptides include IgG, IgE, IgM, IgD, either individually or in a combination with one another.

[0134] Other suitable therapeutic agents include adrenocorticotropic hormone, epidermal growth factor, platelet-derived growth factor (PDGF), prolactin, luliberin, luteinizing hormone releasing hormone (LHRH) agonists, LHRH antagonists, gastrin, tetragastrin, pentagastrin, urogastrone, secretin, enkephalins, endorphins, angiotensins, tumor necrosis factor (TNF), nerve growth factor (NGF), heparinase, bone morphogenic protein (BMP), hANP, glucagon-like peptide (GLP-1), interleukin-11 (IL-11), VEG-F, recombinant hepatitis B surface antigen (rHBsAg), renin, bradykinin, bacitracins, polymyxins, colistins, tyrocidine, gramicidins, and synthetic analogues, modifications and pharmacologically active fragments thereof, enzymes, cytokines, antibodies and vaccines.

[0135] The term dextran microparticles includes unsubstituted dextran microparticles and substituted dextran microparticles. For example, substituted dextran microparticles include dextran substituted with a suitable group, such as a methyl group, up to a degree which does not hamper crystallization of the dextran microparticles, such as up to 3.5 or less percent branching. The average microparticle diameter is preferably about 0.5 to about 5 microns, more preferably about 1 to about 2 microns.

[0136] Furthermore, while porous non cross-linked dextran microparticles, such as crystallized microparticles, are preferably used with the therapeutic agent, other suitable organic or inorganic microparticles may be used instead, such as other polymer microparticles including polysaccharides, PLA, PLGA, PMMA, polyimides, polyesters, acrylates, acrylamides, vinyl acetate or other polymeric materials, biomaterial particles such as alginate and cells, or inorganic particles, such as silica, glass or calcium phosphates. Preferably the microparticles are biodegradable. Preferably, porous microparticles are used. Most preferably, the microparticles have sufficient porosity to contain the therapeutic agent within the pores and to provide a timed release of the therapeutic agent from the pores. In other words, the therapeutic agent is released over time from the pores, such as in over 5 minutes, preferably in over 30 minutes, most preferably in over one hour, such as in several hours to several days, rather than all at once. Thus, the particle material, pore size and pore volume can be selected based on the type of therapeutic agent used, the volume of therapeutic agent needed for delivery, the duration of the delivery of the therapeutic agent, the environment where the therapeutic agent will be delivered and other factors.

[0137] Thus, in a preferred aspect of the present invention, the therapeutic agent is located at least partially in the pores of the porous microparticles. Preferably, the therapeutic agent is not encapsulated in the microparticle (i.e., the microparticle does not act as a shell with a therapeutic agent core inside the shell) and is not attached to the surface of the microparticle. However, if desired, a portion of the therapeutic agent may also be encapsulated in a microparticle shell and/or is attached to the surface of the microparticle in addition to being located in the pores of the microparticle. The location of the therapeutic agent in the pores provides an optimum timed release of the therapeutic agent. In contrast, the therapeutic agent attached to the surface of the microparticle is often released too quickly, while the therapeutic agent encapsulated in the microparticle is often not released soon enough and is then released all at once as the microparticle shell disintegrates. In a two phase system, at least 80% of the therapeutic agent is preferably located in a core surrounded by a wall or shell comprising the microparticles.

I. Methods of Making

[0138] The microparticles may be formed by any suitable method. Preferably, the microparticles are combined with the therapeutic agent after the microparticles are formed. Thus, the microparticles, such as the crystallized dextran microparticles are formed by any suitable method and then the therapeutic agent and the microparticles are combined by any suitable method. In contrast, in some prior art methods, the therapeutic agent is encapsulated into a microparticle shell by providing the particle precursor material and the therapeutic agent into a solution and then crystallizing or cross-linking the precursor material, such as a monomer or oligomer material, to encapsulate a therapeutic agent core into a microparticle shell.

[0139] Preferably, the therapeutic agent is provided into the pores of the porous microparticles after the microparticles are formed. Thus, the porous microparticles are first formed and then the therapeutic agent is provided into a solution containing the microparticles to allow the therapeutic agent to permeate into the pores of the microparticles. Of course, some of the therapeutic agent may also become attached to the surface of the microparticle in this process.

[0140] Thus, a method to manufacture non cross-linked, porous crystallized dextran microparticles includes preparation of a dextran solution, such as an aqueous dextran solution, conducting a crystallization process to form crystallized porous dextran microparticles, and if desired, isolating crystallized porous dextran microparticles from the solution. A therapeutic agent is then permeated into the pores of the microparticles by providing the therapeutic agent into the crystallization solution containing the microparticles or by providing the isolated microparticles and the therapeutic agent into a second solution, such as a second aqueous solution. For example, crystallized dextran microparticles may be formed in a first, low molecular weight dextran aqueous solu-

tion, such as a 2 to 20 kDa dextran solution. The microparticles are then removed from the first solution and then placed into a second dextran aqueous solution having a higher molecular weight dextran, such as 40 to 500 kDa solution, for example a 40 to 75 kDa solution. The second solution may comprise a first phase of a two phase system, which is then combined with a second phase, such as a PEG phase containing a therapeutic agent. A similar method may be used with other porous microparticles, where a therapeutic agent is then permeated into the pores of the microparticles after the porous microparticles are formed by any suitable microparticle formation method, including, but not limited to crystallization. The components of the composition such as insulin, microparticles and one or more aqueous phases may be combined in any suitable order sequentially or simultaneously.

[0141] Preferably, the microparticles are formed by self assembly from a solution that does not contain organic solvents and organic reaction promoters which leave an organic residue in the microparticles. Thus, for example, the dextran microparticles are preferably formed by self assembly from an aqueous dextran solution. However, if desired, organic solvents and/or organic reaction promoters may also be used. In this case, the microparticles may be purified prior to subsequent use to remove the harmful organic residue.

[0142] As described above, the capsule structure having a first phase core and a second phase wall or shell may be formed in vivo or in vitro from a two phase composition. The composition may be a dried powder, such as freeze dried and stored as a powder or porous cake. When the composition is ready to be administered to a mammal, it is hydrated and administered to a mammal orally or by injection.

[0143] Preferably, the composition which includes the microparticles and the therapeutic agent is a flowable colloidal system when the composition is dosed for injection. Examples of flowable colloidal systems include emulsions and suspensions which may be injected into a mammal using a common gage syringe or needle without undue difficulty. In contrast, some prior art compositions include a therapeutic agent in a dextran hydrogel or in a cross-linked dextran matrix. A dextran hydrogel and a cross-linked dextran matrix are not flowable compositions if not specifically prepared.

[0144] In another preferred aspect of the present invention, the microparticles comprise microparticles which are adhesive to mammalian mucosa. Preferably the adhesive microparticles are porous microparticles described above. This further improves the effective delivery of the therapeutic agent.

[0145] In another preferred aspect of the present invention, the microparticles comprise microparticles whose surface has been specially modified to enhance the adhesion of the therapeutic agent to the microparticle surface and to optimize the delivery of the therapeutic agent. The microparticle surface may contain any suitable modification that would increase the adhesion of the therapeutic agent.

[0146] The foregoing description of the invention has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed, and modifications and variations are possible in light of the above teachings or may be acquired from practice of the invention. The drawings and description were chosen in order to explain the principles of the invention and its practical application. It is intended that the scope of the invention be defined by the claims appended hereto, and their equivalents.

[0147] All of the publications and patent applications and patents cited in this specification are herein incorporated in their entirety by reference.

I claim:

1. A pharmaceutical flowable composition comprising biodegradable and biocompatible components for being provided within or upon a mammal body and adapted for forming a biodegradable, biocompatible, three dimensional structured object, the composition comprising a multiphase, flowable colloidal system and therapeutic agent(s) to provide local or systemic therapeutic effect(s) in or upon said body.

2. A pharmaceutical composition for being provided within or upon an mammal body, comprising:

a first phase;

a second phase;

first molecules or molecular aggregates which are adapted to preferentially partition into the first phase; and

second molecules or molecular aggregates which are adapted to preferentially partition into the second phase;

wherein the second molecules or molecular aggregates self assemble a wall adjacent the first molecules or molecular aggregates when the composition is provided into said mammal body.

3. The composition of claim **1**, wherein the colloidal system is a liquid suspension or emulsion containing microparticles.

4. The composition of claim **2**, wherein:

the first molecules or molecular aggregates comprise a therapeutic agent;

the second molecules or molecular aggregates comprise micro particles;

the wall comprises a microparticle shell around a therapeutic agent containing core.

5. The composition of claim **3** or **4**, wherein the microparticles are porous microparticles and wherein the composition is provided by injection into the mammal body.

6. The composition of claim **5**, wherein the porous microparticles are polymer microparticles.

7. The composition of claim **6**, wherein porous microparticles are crystallized dextran microparticles having an average diameter ranging from 0.5 to 5.0 microns.

8. The composition of any claims **1** to **7**, wherein the composition is a liquid two-phase system containing a mixture of microparticles.

9. The composition of claim **8**, wherein the two-phase system forms a capsule structure when provided in vivo, the capsule structure comprising a first phase core and a second phase shell surrounding the core.

10. The composition of claim **9**, wherein the therapeutic agent selectively partitions into the core liquid phase and the microparticles selectively partition into the shell liquid phase.

11. The composition of any claims **8** to **10**, wherein the therapeutic agent is selected from a group consisting of a peptide, a protein, a nucleic acid, a virus, a cell and a combination thereof.

12. The composition of any claims **9** to **11**, wherein the core comprises a PEG aqueous phase and selectively partitioned insulin and the shell comprises a dextran aqueous phase and selectively partitioned crystallized dextran microparticles.

13. The composition of claim **2**, wherein:

the composition comprises a flowable composition or a dried composition which may be rendered flowable by hydration;

the first molecules or molecular aggregates are selected from a group consisting of microparticles, macromolecules, cells, liposomes, DNA, plasmids and proteins; and

the second molecules or molecular aggregates are selected from a group consisting of microparticles, macromolecules, cells, liposomes, DNA, plasmids and proteins.

14. A method of in vivo formation of a biocompatible implant comprising:

forming a pharmaceutical flowable composition comprising a multiphase colloidal system and a therapeutic agent;

injecting the pharmaceutical flowable composition into an mammal body; and

forming a biodegradable and biocompatible structured implant by self assembly in the mammal body.

15. A method of providing a pharmaceutical composition within or upon an mammal body, comprising:

providing the pharmaceutical composition comprising:

a first phase;

a second phase;

first molecules or molecular aggregates which are adapted to preferentially partition into the first phase; and

second molecules or molecular aggregates which are adapted to preferentially partition into the second phase; and

providing the pharmaceutical composition into or upon the mammal body wherein the second molecules or molecular aggregates self assemble a wall adjacent the first molecules or molecular aggregates when the composition is provided into or upon said mammal body.

16. The method of claim **14**, wherein the colloidal system is a liquid suspension or emulsion containing microparticles.

17. The method of claim **15**, wherein:

the first molecules or molecular aggregates comprise a therapeutic agent;

the second molecules or molecular aggregates comprise microparticles;

the wall comprises a microparticle shell around a therapeutic agent containing core.

18. The method of claim **16** or **17**, wherein microparticles are polymer microparticles and the mammal body comprises a human or an animal body.

19. The method of claim **18**, wherein the polymer microparticles are porous microparticles.

20. The method of claim **19**, wherein porous microparticles are crystallized dextran microparticles having an average diameter ranging from 0.5 to 5.0 microns.

21. The method of any claims **14** to **20**, wherein the composition is a two-phase system containing microparticles.

22. The method of claim **21**, wherein the two-phase system forms a capsule structure when provided in vivo, the capsule structure comprising a first phase core and a second phase shell surrounding the core.

23. The method of claim **22**, wherein the therapeutic agent is selectively partitioned in the core and the microparticles are selectively partitioned in the shell.

24. The method of any claims **21** to **23**, wherein the therapeutic agent is selected from a group consisting of a peptide, a protein, a nucleic acid, a virus, and a cell.

25. The method of any claims **22** to **24**, wherein the core comprises a PEG aqueous phase and selectively partitioned insulin and the shell comprises a dextran aqueous phase and selectively partitioned crystallized dextran microparticles.

26. The method of claim **15**, wherein:

the composition comprises a flowable composition or a dried composition which is rendered flowable by hydration prior to being provided into the mammal body;

the first molecules or molecular aggregates are selected from a group consisting of microparticles, macromolecules, cells, liposomes, DNA, plasmids and proteins; and

the second molecules or molecular aggregates are selected from a group consisting of microparticles, macromolecules, cells, liposomes, DNA, plasmids and proteins.

27. A flowable composition, comprising a colloidal suspension or emulsion of biocompatible and biodegradable microparticles and a label in a fluid.

28. The composition of claim **27**, wherein:

the microparticles comprise crystallized dextran microparticles;

the label comprises a fluorescent macromolecule which controllably releases from the composition when it is located in a mammal body.

29. The composition of claim **27**, wherein the colloidal suspension comprises a capsule having a first phase shell comprising selectively partitioned label and a second phase core comprising selectively partitioned microparticles.

30. A method of in vivo formation of a biocompatible implant, comprising:

providing a composition comprising a colloidal suspension or emulsion of biocompatible and biodegradable microparticles and a label in a fluid; and

introducing the pharmaceutical composition into a mammal body.

31. The method of claim **30**, wherein:

the microparticles comprise crystallized dextran microparticles;

the crystallized dextran microparticles are biocompatible with the mammal body and are biodegradable within the mammal body; and

the label comprises a fluorescent macromolecule.

32. The method of claim **30**, wherein the colloidal suspension self assembles into a capsule having a first phase core comprising selectively partitioned label and a second phase shell comprising selectively partitioned microparticles when the suspension is introduced into the mammal body.

33. A cell therapy method comprising providing cells enclosed in or contacting an outer surface of a capsule of crystallized dextran microparticles into a mammal in need thereof.

34. The method of claim **33**, wherein:

the cells are enclosed in the capsule such that the cells comprise a core of the capsule and the microparticles comprise a shell enclosing the core; and

the shell is porous to oxygen and nutrients but impermeable to immune system cells.

35. The method of claim **34**, wherein the cells comprise insulin producing cells which generate and release insulin through the capsule into blood of a mammal to lower blood glucose in response to blood glucose permeation into the capsule.

36. The method of claim **34**, wherein the cells comprise the cells that are not from the mammal to which the composition is being administered to.

37. The method of claim **33**, wherein:
the cells contact the outer surface of the capsule; and
the cells comprise the cells from the mammal to which the composition is being administered to.

38. A bone graft substitute comprising porous crystallized dextran microparticles.

39. The bone graft substitute of claim **38**, further comprising a therapeutic agent located in pores of the microparticles which provides bone formation in special sites of a body.

40. A method of forming a bone graft substitute, comprising providing porous crystallized dextran microparticles to a bone graft site in a mammal body and forming a bone graft substitute comprising the porous crystallized dextran microparticles.

41. The method of claim **40**, further comprising:
preparing a suspension of porous crystallized dextran microparticles in a first liquid phase;
preparing a suspension of second microparticles in a second liquid phase immiscible with the first liquid phase;
preparing an emulsion where the second liquid phase is a continuous phase and the first liquid phase is a dispersed phase; and
injecting of the emulsion into a mammal body.

42. The method of claim **41**, wherein:
the second microparticles comprise ceramic microparticles; and
a therapeutic agent which provides bone formation in special sites of the body is located in pores of the porous crystallized dextran microparticles.

43. A method, comprising:
providing crystallized dextran microparticles; and
combining a therapeutically effective amount of insulin and the crystallized dextran microparticles in a solution after the microparticles have been crystallized to form a suspension of insulin and crystallized dextran microparticles.

44. The method of claim **43**, further comprising administering the suspension to a mammal.

45. The method of claim **44**, wherein:
the crystallized dextran microparticles comprise microparticles having an average diameter of 0.5 to 5 microns;
the step of providing the microparticles comprises forming the microparticles in a non organic solvent;
the solution comprises an aqueous solution; and
the mammal comprises a human.

46. The method of claim **44**, further comprising drying the suspension to provide a composition comprising the crystallized dextran microparticles and the insulin.

47. A method of manufacturing non cross-linked crystallized porous dextran microparticles, comprising:
(a) preparing an aqueous dextran solution that lacks an organic solvent;
(b) conducting a crystallization process to form the dextran microparticles at a temperature above room temperature; and
(c) isolating the non cross-linked crystallized porous crystallized dextran microparticles from the solution.

48. The method of claim **47**, wherein:
the dextran solution comprises dextran having a molecular weight of 2 to 200 kDa; and
the crystallization process is conducted at a temperature of 40 to 99° C.

49. The method of claim **48**, wherein:
the microparticles are spontaneously formed from the solution;
the dextran solution comprises dextran having a molecular weight of 20 to 75 kDa; and
the crystallization process is conducted at a temperature of 40 to 70° C.

50. The method of claim **47**, further comprising combining the microparticles with insulin.

51. A method of making porous crystallized dextran microparticles containing a therapeutic agent in microparticle pores, comprising:
providing a suspension comprising formed porous crystallized dextran microparticles; and
providing the therapeutic agent into the suspension such that the therapeutic agent permeates into pores of the porous microparticles.

52. The method of claim **51**, wherein the step of providing a suspension comprises:
preparing aqueous dextran solution;
conducting a crystallization process to form porous crystallized dextran microparticles;
isolating the microparticles from the solution; and
providing the microparticles into a colloidal system comprising the therapeutic agent.

53. The method of claim **52**, wherein:
the therapeutic agent comprises insulin; and
the colloidal system comprises a suspension comprising insulin and the microparticles.

54. A composition, comprising:
insulin; and
porous crystallized dextran microparticles;
wherein the microparticles are formed prior to combination of the insulin and the microparticles in the composition.

55. The composition of claim **54**, wherein the composition comprises a flowable colloidal composition and the microparticles comprise microparticles having an average diameter of 0.5 to 5 microns.

56. The composition of claim **54**, wherein the insulin is located in pores of the crystallized dextran microparticles but is not encapsulated inside each microparticle.

57. The composition of claim **54**, wherein the insulin is selectively partitioned in a first polymer phase and the microparticles are selectively partitioned in a second polymer phase, such that the composition forms a structured implant upon introduction into a mammal body.

58. A composition, comprising:
insulin;
a first polymer;
a second polymer which is incompatible with the first polymer; and
microparticles.

59. The composition of claim **58**, wherein the composition comprises a flowable colloidal composition and the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns.

60. The composition of claim **59**, wherein the first polymer comprises a dextran and the second polymer comprises PEG.

61. The composition of claim **60**, wherein the insulin is selectively partitioned in a PEG phase and the microparticles are selectively partitioned in a dextran phase, such that the composition forms a structured implant comprising a PEG phase core and a dextran phase shell upon introduction into a mammal body.

62. A method of lowering blood glucose in a mammal, comprising providing a therapeutically effective amount of a composition comprising crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal, wherein the microparticles are formed prior to combination of the insulin and the microparticles in the composition.

63. The method of claim **62**, wherein the composition comprises a flowable colloidal composition and the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns.

64. The method of claim **63**, wherein:

the composition comprises a two phase composition comprising a dextran phase and a PEG phase;

the insulin is selectively partitioned in the PEG phase and the microparticles are selectively partitioned in the dextran phase; and

the composition forms a structured implant comprising a PEG phase core and a dextran phase shell after introduction into a mammal body.

65. The method of claim **64**, further comprising controlling a thickness of the shell based on the body of the mammal receiving the composition to control release of insulin from the implant.

66. The method of claim **62**, wherein the composition is provided to a human suffering from diabetes to lower the blood glucose concentration in the human.

67. An inhalator, comprising:

a vessel adapted for administering a dose of a pharmaceutical composition to a mammal by inhalation; and

a pharmaceutical composition comprising crystallized dextran microparticles and a therapeutically effective amount of insulin located in the vessel.

68. A method of lowering blood glucose in a mammal, comprising administering by inhalation a therapeutically effective amount of a composition comprising crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal.

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