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54. **TÍTULO** Sistema de suministro para drogas y terapia celular.

51. **CLASIFICACIÓN INTERNACIONAL** A61K 38/28, 47/36, 9/00

71. **SOLICITANTE** The Technology Development Company Ltd.

DOMICILIO Hamilton, Bermuda.

74. **APODERADO** Delia María Rodríguez D' Aleman

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FORMULARIO ÚNICO DE SOLICITUD DE PATENTE PCT ENTRADA EN FASE NACIONAL

1) SOLICITUD DE:

☒ Patente de invención ☐ Patente de Modelo de Utilidad

☐ Capitulo I. ☒ Capitulo II.

2) SOLICITANTE (71)	**Nombre: THE TECHNOLOGY DEVELOPMENT COMPANY LTD.** **Dirección: Reid House, 31 Church Street, Hamilton HM FX, Bermuda** **Nacionalidad o domicilio: Hamilton, Bermuda** **Lugar de constitución: Bermuda**	**IDENTIFICACIÓN** **C.C.** ☐ **NIT:** ☐ **C.E.** ☐ **OTRO** ☐ **Cual** _____ **NÚMERO** _____
	Teléfono: Fax: **E. Mail:**	
3) REPRESENTANTE O APODERADO	Nombre: DILIA MARÍA RODRÍGUEZ D´ALEMAN Dirección: Carrera 11 N°. 86-53 Piso 6 Bogotá D.C. Teléfono: 6181088 Fax: 6350824 E. Mail: info@clarkemodet.com.co	**IDENTIFICACIÓN** **C.C.** ☒ **NIT:** ☐ **C.E.** ☐ **OTRO** ☐ **NÚMERO:** 41.654.882 **TP:** 20824 CSJ
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Instrucciones para el diligenciamiento del presente formulario. Ver reverso de esta pagina

10) ANEXOS

- [x] Comprobante de pago de la tasa de presentación de la solicitud
- [ ] Comprobante de pago por reivindicación de prioridad
- [ ] Documento que acredita la existencia y representación legal cuando el solicitante sea persona Jurídica
- [ ] Poderes, si fuera el caso
- [ ] Documento de cesión del inventor al solicitante o a su causante
- [ ] Declaraciones
- [x] Copia de la solicitud Internacional. (Resumen, descripción, reivindicación, Dibujos).
- [x] Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicación, Dibujos)
- [ ] Copia del examen preliminar efectuado por la IPEA
- [ ] De ser el caso, copia del contrato de acceso
- [ ] De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas
- [ ] De ser el caso, certificado de depósito del material biológico
- [ ] Arte final 12 x 12 y 6 x 6 cm
- [ ] De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionados parcial o totalmente con la invención de esta solicitud.
- [x] Otros:
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11) FIGURA CARACTERÍSTICA:

12) Solicitud concesión de la patente,

NOMBRE: DILIA RODRÍGUEZ D´ALEMAN

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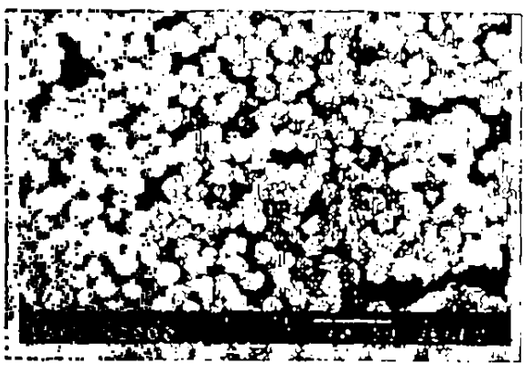
(54) Title: DELIVERY SYSTEM FOR DRUG AND CELL THERAPY



(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.

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DELIVERY SYSTEM FOR DRUG AND CELL THERAPY

FIELD OF THE INVENTION

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

[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 theraples 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

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

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

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

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

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

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

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

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

[0016] Figure 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 14th day after intermuscular injection.

[0017] Figure 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] Figure 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 Figure 1.

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

[0020] Figure 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 Figure 1.

[0021] Figure 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 Figure 1.

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

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

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

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

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

[0027] Figures 22A and 22B 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 Figures 2A, 2B). 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 Figure 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 microparticles, 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] Figures 4 and 5 show the implant in tissue following subcutaneous (Figure 4) and intra-muscular (Figure 5) injections in experimental animals (mice). No inflammation reactions were detected in the animal's tissue during 180 days.

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

[0036] Figures 8A, 8B and 8C show the implant on the 4th, 11th and 28th day, respectively, after the subcutaneous injection. Figures 8D and 8E both show the implant 180 days after the subcutaneous injection. Figures 8F and 8G both show the implant one year after the

subcutaneous injection. As shown in Figure 8G, 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] Figures 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 14th day after the intermuscular injection (Figure 9) and expression of the reporter gene in a mouse's muscle tissue following the plasmid DNA release from the implant (Figure 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 surrounded with a shell composed of crystallized dextran microparticles or aggregates thereof dispersed in water or aqueous solutions of organic polymers such as

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. Patent 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. Patent 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

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, publs. 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 Figures 11 and 12.

[0045] Figure 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 Figure 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] Figure 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 Figure 12, the smaller volume (droplet) dextran phase forms into a large spherical dextran / dextran microparticle core (bottom right of Figure 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 Figures 13 and 14. The volume of the dextran phase is greater than the volume of the PEG phase in the two-phase system. Both Figures 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. Figure 15A 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 Figure 15A. 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 Figure 15A, 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 Figure 15A, 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] Figure 15B illustrates a scanning electron microscope image of a cross section of an implant structure based on the two phase system schematically illustrated in Figure 15A. 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 Figure 15B 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 Figure 15B forms by self assembly as shown schematically in Figure 15C. 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 Figure 15C. 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 Figure 15C. 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 Figures 3 and 12) or absorbed by the same surface being inside of macro-porous structures (see Figure 11) or capsules (see Figures 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, Figures 16A-16D illustrate a partition of HeLa cells and crystallized dextran microparticles in a dextran-PEG two phase system. In Figure 16A, 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. Figure 16B 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. Figures 16C and 16D 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] Figure 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 Figure 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 Figure 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 Figure 18. Furthermore, as shown in Figure 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 Figure 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,

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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

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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 Figure 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. Figure 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, CA, 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

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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	98	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	69	68	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 GL, Lee HJ, 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 Figure 19, a two phase composition, described above and illustrated in Figures 13, 14, 15A, 15B, 15C and

16B 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 Figure 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. Figure 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. Figure 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, MA) 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 x 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, CA, 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 #		0min glucose mmol/l	15min glucose mmol/l	30min glucose mmol/l	45min glucose mmol/l	120min glucose mmol/l	210min glucose mmol/l	270min glucose mmol/l	360min 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 crystal-lized dextran micro-particles	6.0	4.3	3.2	2.5	0.8	0.8	0.9	0.7
19	Insulin 0.5 UI with crystal-lized dextran micro-particles	6.9	5.6	4.1	3.4	-	1.2	-	1.6
20	Insulin 0.5 UI with crystal-lized dextran micro-particles	5.9	3.5	2.9	1.9	1.2	1.0	1.0	0.7
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 Figures 22A and 22B, 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 (Figure 22B) and about seventeen to about twenty-four hours for "Monotard HM"® insulin alone (Figure 22A). 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 Figures 22A and 22B, the upper line illustrates the control line for intact rabbits to which no insulin was administered. The y-axis of Figures 22A and 22B 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 Figures 22A and 22B 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, CA, 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	5.6
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	2.8	2.5	2.4	4.0	5.1	N.A
27	8 UI	5.8	3.7	1.9	1.8	1.8	2.8	4.1	4.3	4.1
28	8 UI	6.6	5.7	4.3	3.8	3.7	3.8	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/Pis/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 (Fluka) 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, semi-synthetic or recombinant amylin or amylin analogs including pramlintide and other amylin agonists.

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[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, goserelin, 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.

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[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

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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, lulliberin, 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

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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 solution, 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 microparticles;

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

5. The composition of claims 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 claims 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.

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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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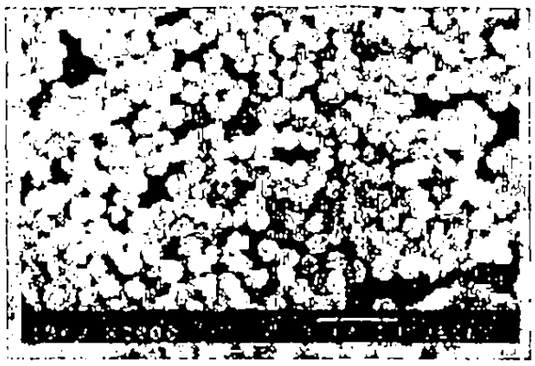

FIG. 1

FIG. 2A

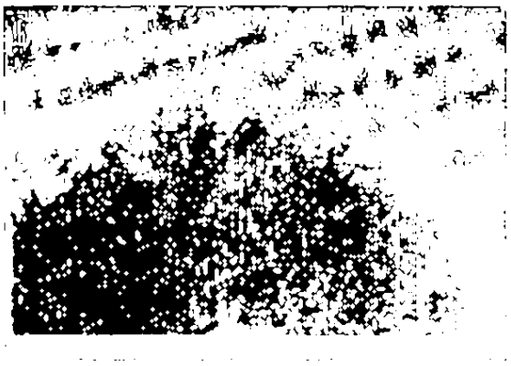

FIG. 2B

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73

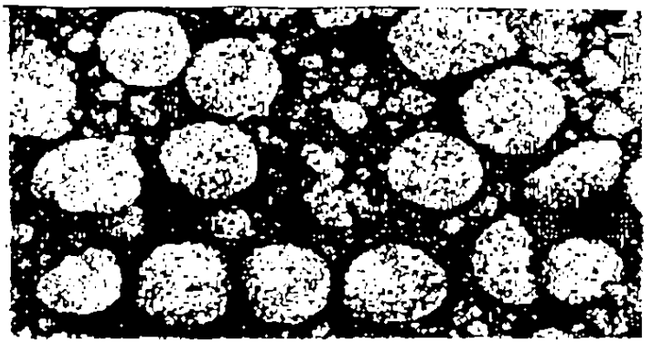

FIG. 3

FIG. 4

FIG. 5

74

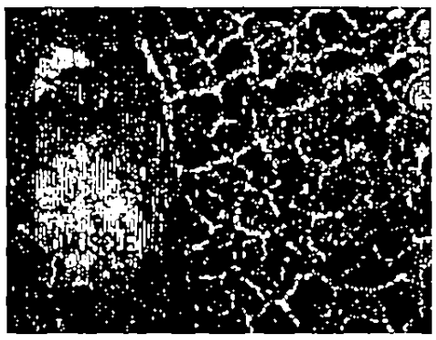

FIG. 6A

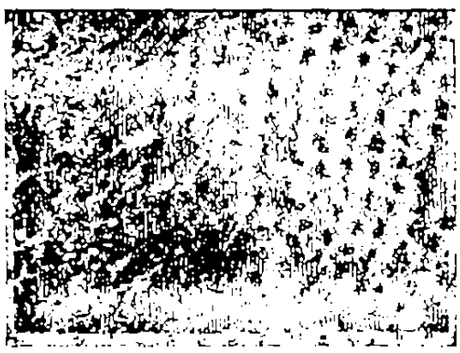

FIG. 6B

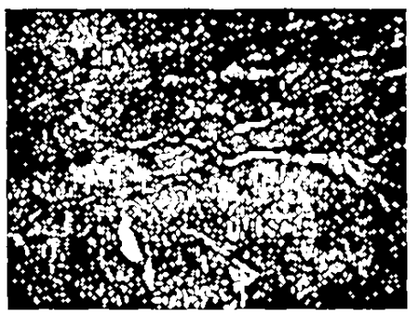

FIG. 6C

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FIG. 7A

FIG. 7B

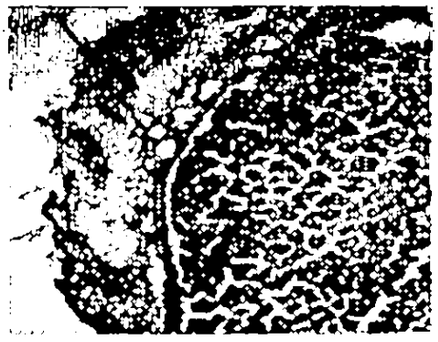


FIG. 8A

FIG. 8B

FIG. 8C

FIG. 8D

FIG. 8E

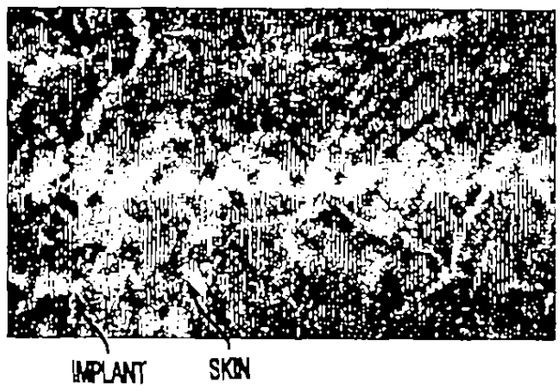


FIG. 8F

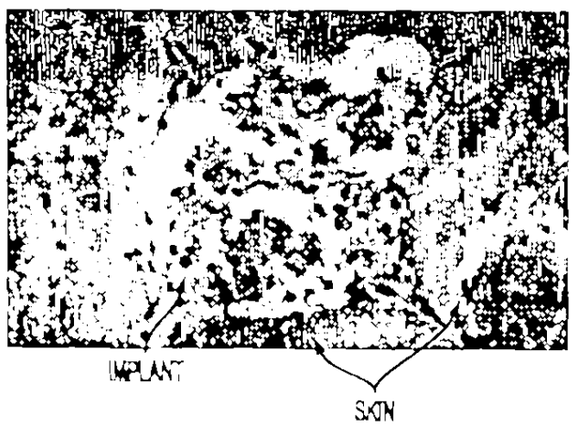


FIG. 8G

FIG. 9

FIG. 10

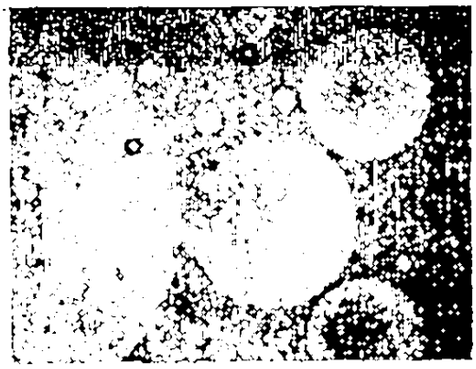


FIG. 11

FIG. 12

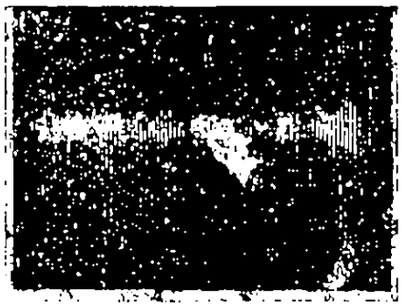
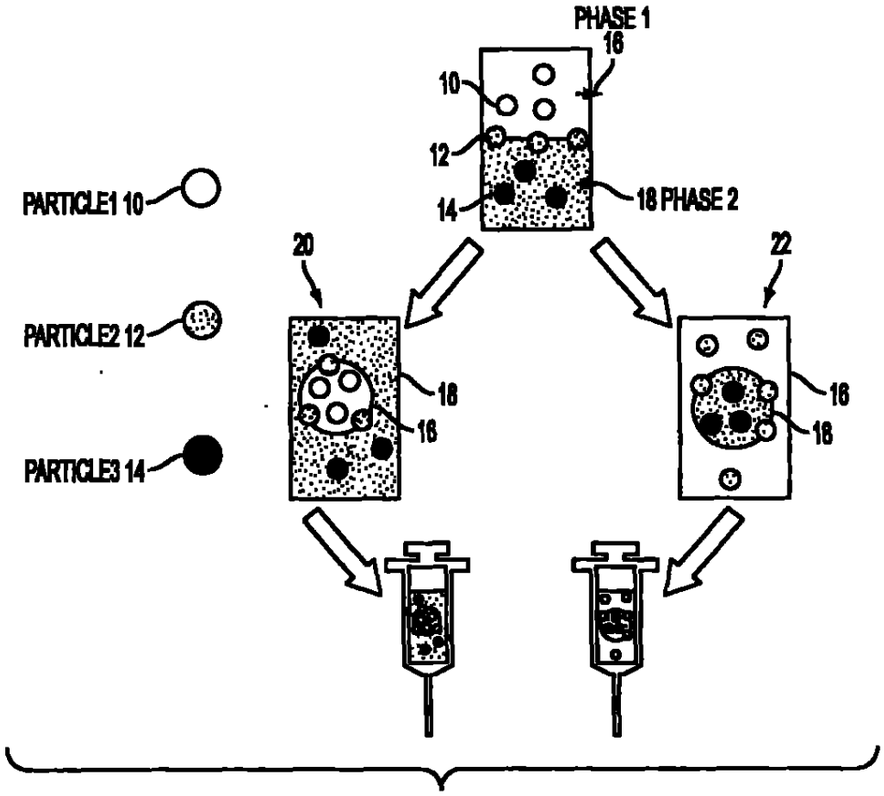


FIG. 13

FIG. 14


PHASE 1
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18 PHASE 2
PARTICLE1 10
PARTICLE2 12
PARTICLE3 14
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FIG. 15A

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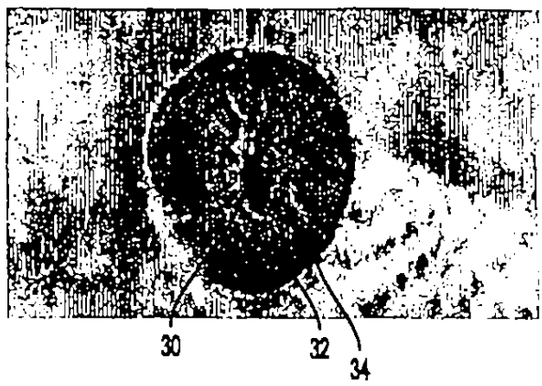


FIG. 15B

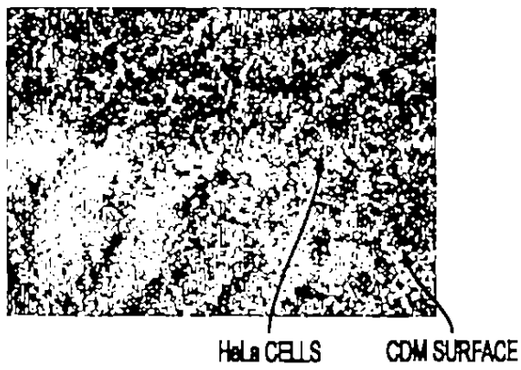


FIG. 16A

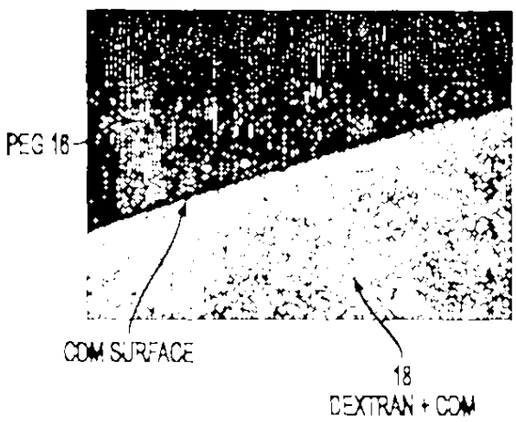
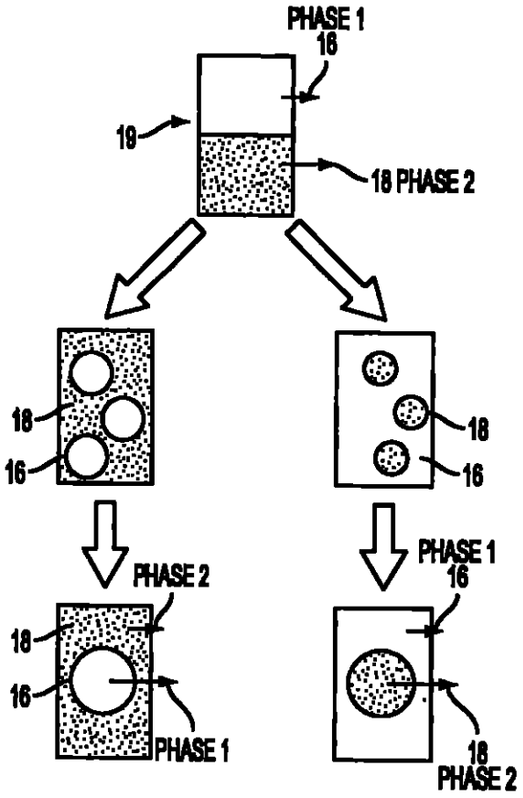


FIG. 16B


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

FIG. 15C

83

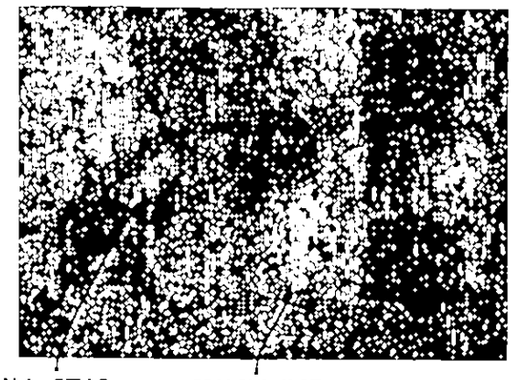


FIG. 16C

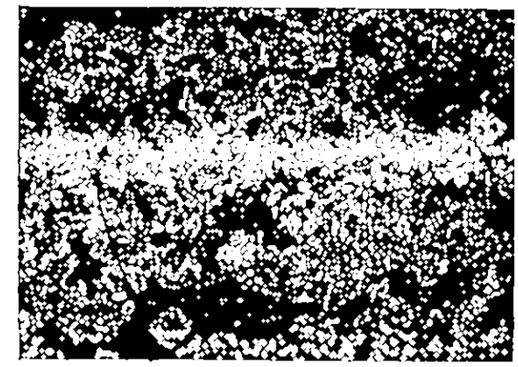
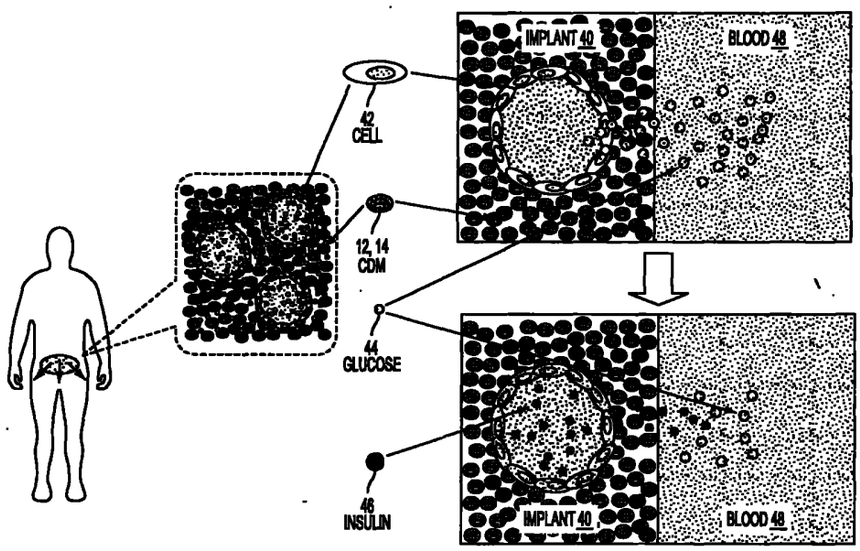


FIG. 16D


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

FIG. 17

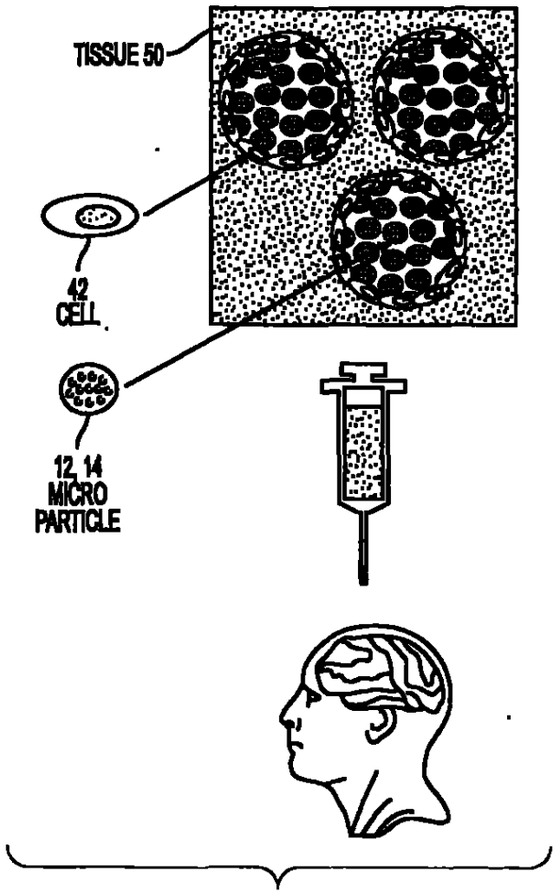

FIG. 18

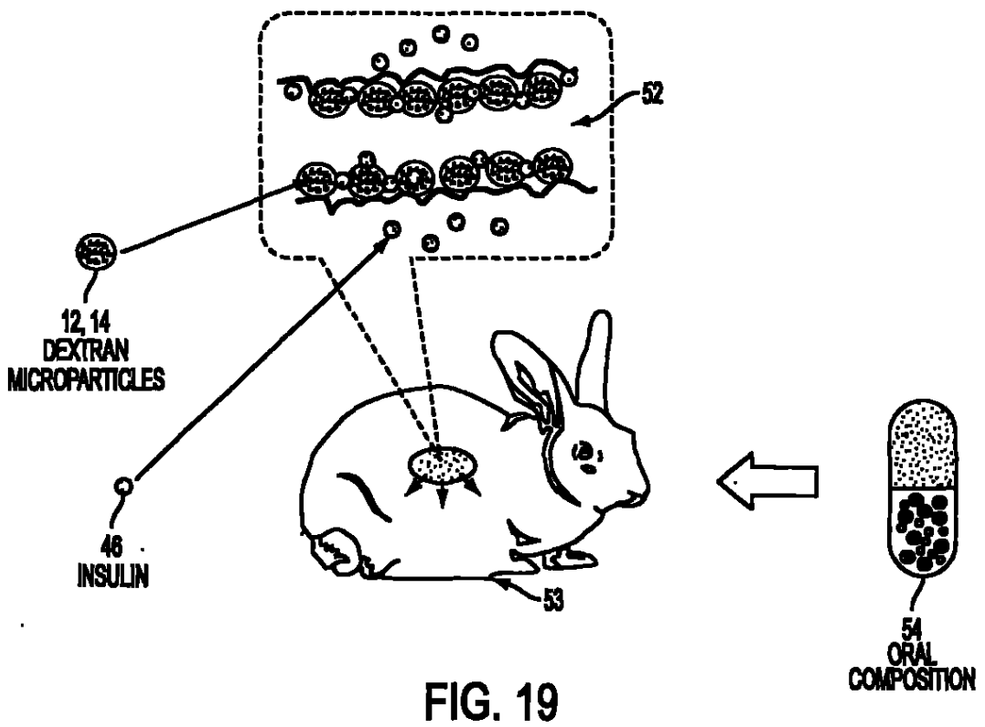
52
12, 14
DEXTRAN
MICROPARTICLES
46
INSULIN
53
54
ORAL
COMPOSITION

FIG. 19

87

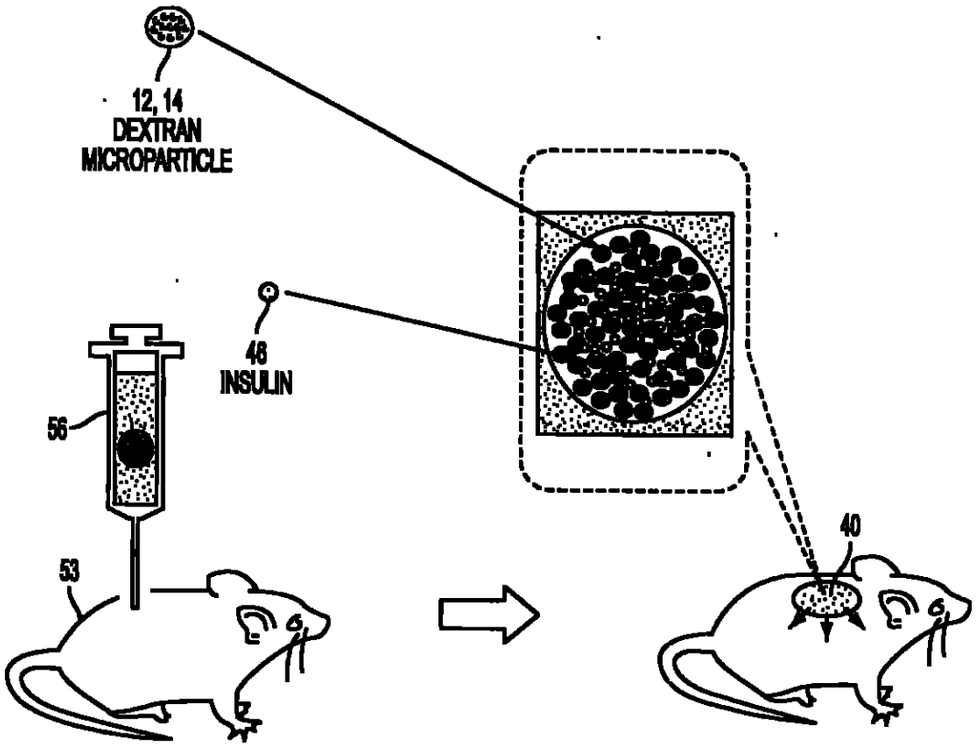

FIG. 20

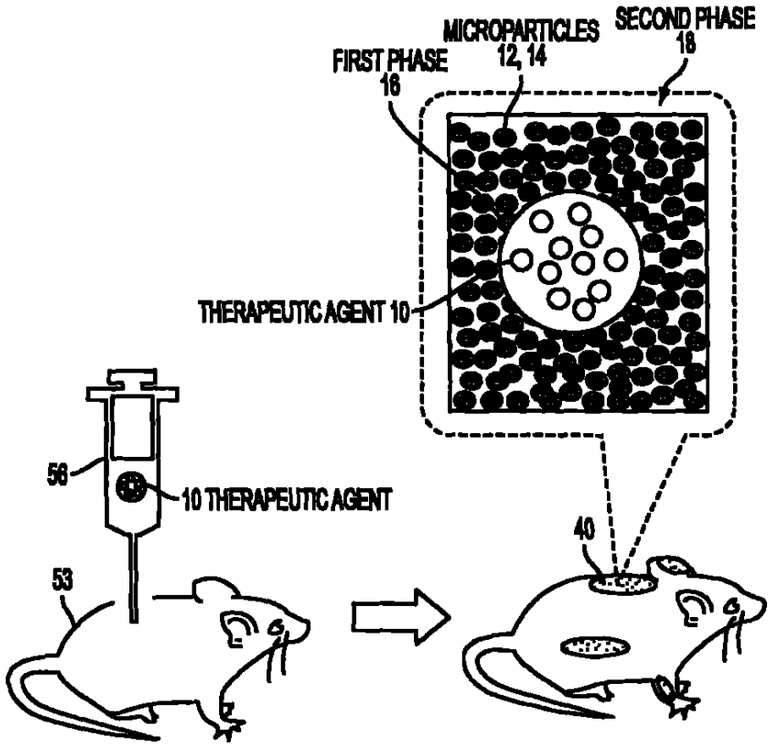
MICROPARTICLES
12, 14
SECOND PHASE
18
FIRST PHASE
16
THERAPEUTIC AGENT 10
56
10 THERAPEUTIC AGENT
53
40

FIG. 21

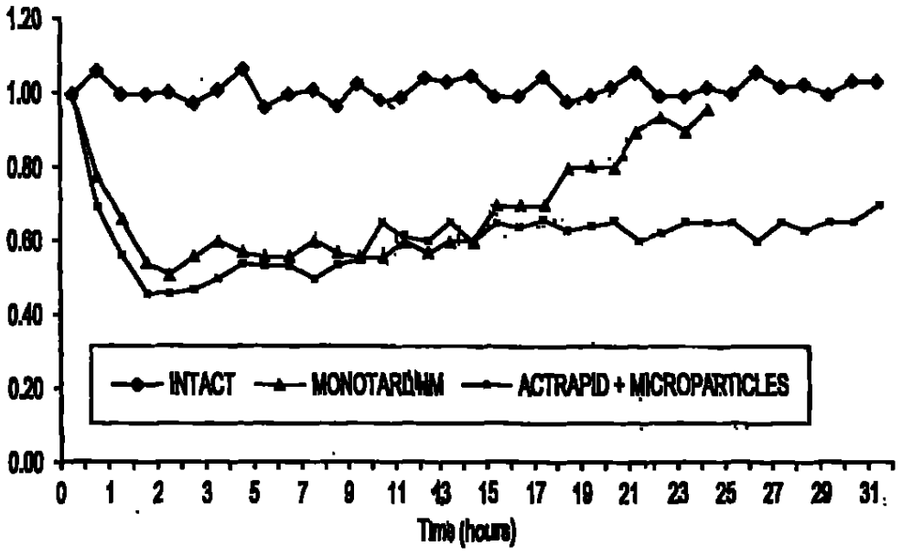

FIG. 22A

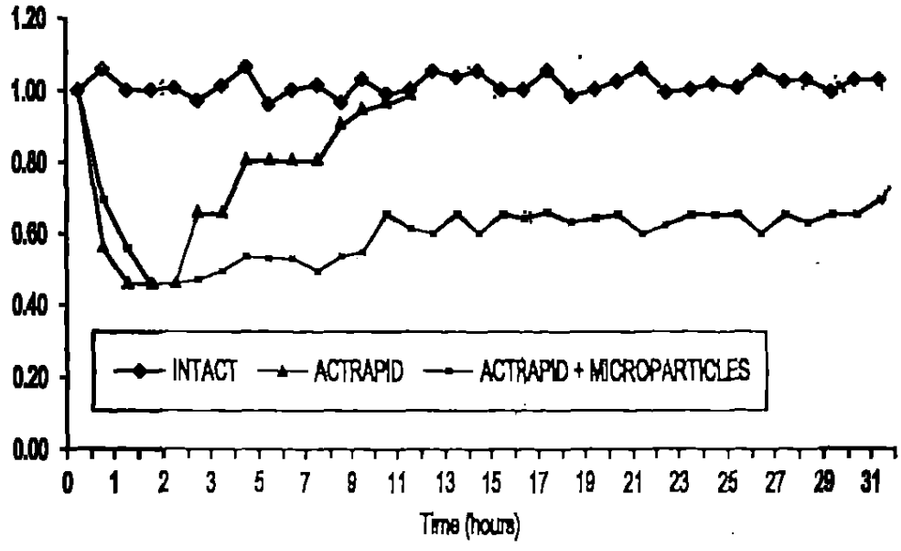

FIG. 22B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB2004/000961

46
90

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/28 A61K47/36 A61K9/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/39985 A (LAAKSO TIMO ; RESLOW MATS (SE); BIOGLAN AB (SE); JOENSSON MONICA (SE)) 23 May 2002 (2002-05-23) page 19, line 21 - page 20, line 10 page 19, line 29 - line 36	1-11,13
X	WO 02/28374 A (BRYSON NATHAN ; FLAMEL TECH SA (FR)) 11 April 2002 (2002-04-11) page 1, line 16 - line 21 page 9, line 14 - line 27	1
X	US 4 963 526 A (ECANOW BERNARD) 16 October 1990 (1990-10-16) claims 1,25	1,2

-/—

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

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

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 July 2004	27/07/2004

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

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Form PCT/ISA/210 (second sheet) (January 2004)

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MORIYAMA K ET AL: "Regulated insulin release from biodegradable dextran hydrogels containing poly(ethylene glycol)" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 42, no. 3, 1 December 1996 (1996-12-01), pages 237-248, XP004069859 ISSN: 0168-3659 page 240	1-13, 15-29, 43-61
Y	page 238, column 1 - column 2	62-68
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INTERNATI NAL SEARCH REPORT

International application No.
PCT/IB2004/000961

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Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

Although claims 14-26, 30-37, 40-46, 62-66 and 68 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the composition.

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

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

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

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

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

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

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

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

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB2004/000961

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SISTEMA DE SUMINISTRO PARA DROGA Y TERAPIA CELULAR

CAMPO DE LA INVENCIÓN

[0001] Solicitud revindica el beneficio de las siguientes Solicitudes Provisionales U.S. Serie número 60/451,245, presentada en marzo 4, 2003; 60/467,601, presentada en mayo 5, 2003; 60/469,017 presentada en mayo 9, 2003; y 60/495,097 presentada en agosto 15, 2003, cuya descripciones se incorporan como referencia aquí en su totalidad.

[0002] la presente invención se relaciona de manera general con el desarrollo de biomateriales para el suministro sistémico y local de agente terapéuticos directamente dentro o en los tejidos del cuerpo y para ingeniería de tejido. En particular, la presente invención esta dirigida a la fabricación de implantes biocompatibles y contienen células, sustancias biológicamente activas o combinaciones de estas y con materiales de matriz de micropartícula para suministro de proteínas, péptidos de ácidos nucleicos.

ANTECEDENTES DE LA INVENCIÓN

[0003] El termino "biomaterial" ha sido utilizado alternativamente para describir el material derivado de fuentes biológicas o para describir el material utilizado para terapias en el cuerpo humano. La presente invención está relacionada con el último. El termino "biocompatible" se utiliza aquí para significar que el material en si mismo o una combinación con agentes terapéuticos, que incluyen células vivas, no produce reacción de cuerpo extraño o fibrosis.

[0004] Los dextranos son polisacáridos de alto peso molecular sintetizados por algunos microorganismos o por métodos bioquímicos. El dextrán con un peso molecular promedio de aproximadamente 75 kDa tiene una presión osmótica coloide similar al plasma sanguíneo, así que sus soluciones acuosas son utilizadas químicamente como expandidores de plasma. Los dextranos reticulados en la forma de glóbulos son el o la base para "Sephadex®" que se utiliza en GPC de proteínas y para "Citodex®" desarrollado por farmacia para llenar los requisitos especiales de un cultivo de célula microportadora. Por ejemplo, la patente U.S. números 6,395,302 y 6,303,148 (Inc. Et al.) describe unir varios biomateriales a partículas de dextrán reticuladas. Sin

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embargo, los glóbulos basados en dextrán reticulado generalmente no pueden ser utilizados para elaborar implante debido a su potencial toxicidad debido a la aplicación de agente reticulantes (Blain J.F., Maghni K., Pelletier S. and Sirois P. Inflamm. Res. 48 (1999): 386-392).

[0005] La Patente U.S. número 4,713,249 (Schroder) describe un método de producir una matriz de deposito para sustancias biológicamente activas. De acuerdo a esta patente, la matriz de depósito consiste de acuerdo con lo que se dice de micropartículas de carbohidrato, estabilizadas por cristalización, que implican utilizar uniones no covalentes. El siguiente proceso para producir las micropartículas de carbohidrato cristalizadas de acuerdo con lo dicho se describe por Schroder. Una solución de un carbohidrato polimérico y una sustancia biológicamente activa se forman en uno o más solventes hidrofílicos. Entonces la mezcla de carbohidrato en la sustancia biológicamente activa se emulsifica en un medio hidrófobo o líquido para formar gotas esféricas. La emulsión es luego introducida en un medio cristalizante que comprende acetona, etanol o metanol para formar esferas que tienen matriz de carbohidrato polimérico cristalino no covalentemente reticulado, dicha matriz incorpora 0.001-50% en peso de la sustancia biológicamente activa. Así, la sustancia biológicamente activa se suministra

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en la solución antes de cristalizar las esferas. Schroder no describe la microestructura de las micropartículas hechas por el método multietapa. El método multietapa de Schroder es complejo y utiliza solventes orgánicos que son potencialmente tóxicos a las células y necesitan ser removidos.

BREVE RESUMEN DE LA INVENCIÓN

[0006] Un método para distribuir la glucosa de la sangre en un mamífero incluye administrar oralmente o mediante inyección o inhalación una cantidad terapéuticamente efectiva de micropartícula de dextrán cristalizadas e insulina al mamífero para disminuir la glucosa de la sangre del mamífero. La composición puede ser una composición de una fase o multifase estructurada para la liberación controlada de insulina u otros agentes terapéuticos.

BREVE DESCRIPCIÓN DE LOS DIBUJOS

[0007] La figura 1 es una fotografía de micropartículas de dextrán cristalizadas espontáneamente formadas en una solución acuosa al 55% (P/P) de dextrán con PM 70.0 kDa.

[0008] La figura 2A es una fotografía de una sección transversal de micropartículas de dextrán cristalizadas mostradas en la figura 1.

[0009] La figura 2B es una fotografía de la sección transversal de una micropartícula mostrada en la figura 2A. La estructura microporosa de micropartículas se pueden ver.

[0010] La figura 3 es una fotografía de agregados de micropartículas de dextrán cristalizadas.

[0011] La figura 4 es una fotografía de un implante subcutáneamente inyectado que consiste de micropartículas de dextrán cristalizado mostradas por la figura 3.

[0012] La figura 5 es una fotografía de un implante inyectado intramuscular que consiste de micropartículas de dextrán cristalizadas mostradas en la figura 3.

[0013] Las figuras 6A, 6B y 6C son fotografías de una sección transversal de músculo de ratón con un implante inyectado que consiste de micropartículas de dextrán cristalizadas (el día 1, 4, y 28 después de inyección, respectivamente).

[0014] Las figura 7A y 7B son fotografías de una sección transversal de músculo de ratón con implante inyectado que consiste con micropartículas de dextrán cristalizado (180 días después de inyección).

[0015] Las figuras 8A, 8B, 8C, 8D, 8E, 8F y 8G son fotografías de una sección transversal de una piel de ratón con un implante inyectado y consiste de micropartículas de dextrán cristalizadas (primer día, cuarto día, veintiochoavo día, 180 días, 180 días, un año y un año después de inyección, respectivamente).

[0016] La figura 9 es una fotografía de una liberación lenta de macromoléculas fluorescentemente marcadas con el implante que incluye micropartículas de dextrán cristalizadas en el tejido de músculo de ratón en el catorceavo día después de la inyección intramuscular.

[0017] La figura 10 es una fotografía de una expresión de un gen reportero en un tejido muscular de ratón seguido por la liberación de ADN de plásmido de implante.

[0018] La figura 11 es una fotografía de una emulsión de una solución acuosa de PEG en solución acuosa de dextrán (WM

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500 kDa) que contiene micropartículas de dextrán cristalizadas mostradas en la figura 1.

[0019] La figura 12 es una fotografía de una emulsión de una solución acuosa de dextrán (WM 500 kDa) que contiene micropartículas de dextrán cristalizadas mostradas en la figura 1 en solución acuosa de PEG.

[0020] La figura 13 es una fotografía de una inyección intramuscular de emulsión de solución acuosa de PEG en solución acuosa de dextrán (WM 500 kDa) que contiene micropartículas de dextrán cristalizadas mostradas en la figura 1.

[0021] La figura 14 es una fotografía de una inyección subcutánea de emulsión de solución acuosa de PEG en solución acuosa de dextrán (WM 500 kDa) que contienen micropartículas de dextrán cristalizadas mostradas en la figura 1.

[0022] Las figuras 15A y 15C ilustra esquemáticamente el comportamiento de partición de diferentes tipos de partículas y fases en un sistema de dos fase acuoso.

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[0023] La figura 15B es un fotografía de una sección transversal de una estructura de implante basada en el sistema de dos fases.

[0024] Las figuras 16A, 16B, 16C y 16D son fotografías de partición de células HeLa y micropartículas de dextrán cristalizado en un sistema de dos fases.

[0025] Las figuras 17 y 18 ilustran esquemáticamente métodos de terapia celular de acuerdo con modalidades de la presente invención.

[0026] Las figura 19, 20 y 21 ilustran esquemáticamente métodos de suministro de agente terapéutico de acuerdo con la modalidad de la presente invención.

[0027] Las figuras 22B y 22B son graficas de concentraciones normalizadas relativas de glucosa de la sangre para varias composiciones que contienen insulina versus tiempo.

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DESCRIPCIÓN DETALLADA DE LAS MODALIDADES PREFERIDAS

A. Formación de Micropartícula

[0028] La presente invención ha encontrado experimentalmente las micropartículas cristalizadas de dextrán con un diámetro promedio que varia desde 0.5 a 3.5 micrómetros fueron espontáneamente formadas en soluciones acuosas concentradas con dextranos (40 - 65% P/P) con pesos moleculares que varían desde 1.0 a 200.0 kDa, la temperatura que varia de 20 - 90°C. Si es el deseo formar las micropartículas a temperatura ambiente entonces se pueden utilizar 2 a 18 kDa de soluciones de dextrán. Por supuesto, las micropartículas se pueden formar con soluciones de 2 a 18 kDa a temperaturas por encima de la temperatura ambiente del cuarto, si se desea. Las micropartículas pueden ser espontáneamente formadas de soluciones de dextrán de peso molecular mayor, tal como soluciones de 20 a 75 kDa, a temperaturas mayores por encima de la temperatura ambiente de cuarto, tal como aproximadamente 40 a aproximadamente 70°C. Las micropartículas pueden tener cualquier forma adecuada tal como forma regular o irregular, pero son preferiblemente esféricas de forma y son preferiblemente de 10 micrómetros de diámetro a dos menos, tal como 0.5 a 5 micrómetros.

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[0029] La Microscopia del Electrón de transmisión revelo la estructura micro porosa de las micropartículas de dextrán cristalizado (ver figuras DA, AB). Preferiblemente, la porosidad de la micro partícula es al menos 10% en volumen, tal como aproximadamente 10 a aproximadamente 50%, más preferiblemente aproximadamente 20 a aproximadamente 40%. Así, la estructura comprende micropartículas microporosas con áreas de macroporosidad localizadas entre las partículas.

[0030] El secado por rociado de las suspensiones acuosas de las micropartículas de dextrán cristalizadas a mostrado la posibilidad de producir agregados sustancialmente esféricos de micropartículas de dextrán cristalizado con un diámetro que varia desde 10.0 a 150.0 micrómetros (ver figura 3).

[0031] Un ejemplo no limitante de un método para formar las micropartículas de dextrán es como sigue. 50.0 g de dextrán T40 (peso molecular 40 kDa) de Amersham Biosciences se agrega a 50.0 g de agua destilada estéril en un beaker de laboratorio de 500 ml para obtener una solución 50% P/P bajo flujo laminar. La mezcla se agita a 60°C (baño de agua) con un agitador magnético a 50 rpm hasta que el dextrán es completamente disuelto y se obtiene la solución clara. La solución se le puede poner vacío para remover todas las inclusiones de aire. La solución clara se ubica en el horno

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de laboratorio a 60°C bajo una tapa Tyvek®. 3.5 horas más tarde se desarrolla una suspensión viscosa turbia como resultado de la formación de micropartículas de dextrán cristalizado.

[0032] Para eliminar el estado no cristalizado, las micropartículas son lavadas mediante centrifugación, por ejemplo 3,000 g, 30 min, con 3 X 250 ml de agua estéril destilada, o mediante filtración de suspensión diluida de micropartículas, por ejemplo una parte de micropartículas y 10 partes de agua (3 X 250 ml de agua estéril destilada a través de filtro de esterilización). La centrifugación/lavado es hecho bajo flujo laminal. Las micropartículas son ubicadas en un beaker de laboratorio de 500 ml bajo una tapa Tyvek® secada a 60°C en un horno de laboratorio durante 8 horas para alcanzar un nivel de humedad de aproximadamente 5%. El polvo seco resultante consiste en partículas con un diámetro medio de aproximadamente 2 micrómetros.

B. Implantes basados en micropartículas de dextrán cristalizado.

[0033] Las suspensiones concentradas de las micropartículas de dextrán cristalizadas y los agregados de estos fueron ensayados como de implantes en experimentos con

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ratones para ensayar su biocompatibilidad luego de inyección en el cuerpo del animal.

[0034] Figuras 4 y 5 muestran el implante en el tejido seguido por inyecciones subcutáneas (figura 4) intramuscular (figura 5) el animal es experimental (ratones). No se detectaron reacciones de inflamación en le tejido del animal durante 180 días.

[0035] Figuras 6A, 6B y 6C muestran el implante en el tejido muscular de un ratón 1,4 y 28 días, respectivamente, después de la inyección. Las figuras 7A y 7B muestran ambas el implante en el tejido muscular de un ratón 180 días después de la inyección.

[0036] Las figuras 8A, 8B y 8C muestran el implante en el cuarto, onceavo y ventiochoavo día, respectivamente, después de la inyección subcutánea. Las figuras 8D y 8E muestran ambas el implante de 180 días después de inyección subcutánea. Las figuras 8F y 8G muestran ambas el implante un año después de la inyección subcutánea. Como se muestra en la figura 8G, el tejido normal de las formas de tejido cicatrizados en forma en el sitio del implante.

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[0037] la liberación lenta de macromoleculas de implantes se ha demostrado en experimentos donde las macromoléculas fueron disueltas en suspensiones acuosas de micropartículas de dextrán cristalizadas o sus agregados antes de inyecciones.

[0038] Las figuras 9 y 10 muestran el implante que contienen macromoléculas fluorescentemente marcadas (FITC-dextrán, WM 500 Ada) y liberación lenta de macromoléculas provenientes de implantes en un tejido muscular de ratón al catorceavo día después de la inyección intramuscular (figura 9) y la expresión del gen reportero en un tejido muscular de ratón luego de la liberación de ADN de plásmido proveniente del implante (figura 10). Así, las micropartículas de dextrán cristalizados se pueden utilizadas para liberación temporizada un marcador, tal como un marcador fluorescente solo o en combinación con un agente terapéutico.

C. Los implantes basados en el sistema de dos fases

[0039] Las estructuras autoensambladas de implantes basados en micropartículas de dextrán cristalizado o sus agregados se pueden formar basadas en dos sistemas de fase.

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[0040] Los sistemas coloidales tales como cuotas de aceite, liposomas, micro y nano-partículas se pueden dispersar en una suspensión de micropartículas de dextrán cristalizadas e inyectadas para formar un implante que libera los agentes terapéuticos luego de la administración en el cuerpo del mamífero.

[0041] Por ejemplo, en el caso de aceite, se puede formar una clase de estructura de implante donde el núcleo de aceite esta circundado con una concha compuesta de micropartículas de dextrán cristalizado o agregados de estos dispersados en agua o soluciones acuosas en polímeros orgánicos tales como polisacáridos (por ejemplo dextrano). La estructura descrita se puede diseñar como una cápsula. Se debe notar que la concha puede comprender una concha de forma aproximadamente esférica que resulta cuando la cápsula está circundada por tejido. Sin embargo, cuando la cápsula está localizada cerca de una barrera, tal como un sustrato, hueso o pared de intestino, la cápsula puede comprender un núcleo localizado entre una o más paredes de micropartículas a un lado de la barrera o al otro lado. Adicionalmente, aunque se utiliza aceite como un ejemplo ilustrativo, el núcleo puede comprender otros materiales, tal como otros polímeros, células, etcétera.

[0042] Transformar la estructura de cápsula, son aplicados los sistemas acuosos de dos fases. Cuando las soluciones acuosas de diferentes polímeros son mezcladas por encima de ciertas concentraciones ellas frecuentemente forman soluciones de dos fases no miscibles en líquido. Cada una de las fases usualmente consiste de más de 90% de agua y se puede amortiguar y hacer isotónicas. Si una célula o suspensión de partículas se agrega a tal sistema, las células de la partícula se encuentran frecuentemente que han sido particionadas no igualmente entre fases. El comportamiento de partición preferencial se puede utilizar como una base para los procedimientos de separación para diferentes poblaciones de células o partículas de manera que la partición en estos sistemas se determina directamente mediante propiedades de célula o de superficie de partícula. Las células de las partículas que no tienen propiedades de superficie idénticas exhiben un comportamiento de partición suficientemente diferente.

[0043] La solución competitiva de la fase de dos polímeros depende de la naturaleza química de los polímeros. Un método de polímero de dos fases se ha aplicado para separar o particionar células, proteínas, ácidos nucleicos y minerales ("Partitioning in Aqueous Two-Phase Systems", 1985, eds., H. Walter, D. Brooks, and D. Fisher, publs. Academic Press).

[0044] Los experimentos con la distribución de micropartículas de dextrán cristalizadas en los sistemas de fase privados de, por ejemplo, mezclas de dextrán/polietileno glicol (PEG) revelaron que las micropartículas de dextrán prefieren estar en la fase de dextrán, aunque otra fase PEG puede ser dispersada en la fase de dextrán para formar una emulsión P/P y viceversa en el caso de que el volumen de la fase PEG sea mayor que el volumen de la fase de dextrán, como se muestra en las figuras 11 y 12.

[0045] La figura 11 es una fotografía de una emulsión de una solución acuosa PEG en solución acuosa de dextrán que contiene micropartículas de dextrán cristalizadas. En la estructura de la figura 11, el volumen de la fase de PEG es menor que el volumen de la fase de dextrán. La fase de dextrán contiene el dextrán y las micropartículas de dextrán cristalizadas. Así, la fase PEG se forma en uno o más núcleos con forma de esfera circundada por dextrán/conchas de micropartículas de dextrán (es decir una estructura de poros cerrada).

[0046] La figura 12 es una fotografía de una emulsión de una solución acuosa de dextrán que contiene micropartículas de dextrán cristalizadas en solución acuosa de PEG, donde el

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volumen de la fase de PEG es mayor que el volumen de la fase de dextrán. En este caso, la fase de dextrán se forma en uno o más núcleos con forma de esfera que contienen las micropartículas de dextrán circundadas por fase PEG (es decir una estructura de poro abierta que esta formando en vivo mientras el PEG se disipa en el líquido del tejido). La figura 12 es una fotografía de una emulsión de una solución acuosa de dextrán que contiene micropartículas de dextrán cristalizado de una solución acuosa de PEG, donde el volumen de la fase de PEG es mayor que el volumen de la fase de dextrán. En este caso, la fase de dextrán forma en uno o más núcleos con forma de esfera en este caso, la fase de dextrán se forma en uno o más núcleos con forma de esfera que contienen micropartículas de dextrán secundadas por una fase PEG (es decir una estructura de poro abierta que se forma en vivo mientras el PEG se disipa en líquido de tejido). Como se puede ver en la figura 12, la fase de dextrán de volumen más pequeño (gota) se forma en un núcleo de dextrán esférico grande/micropartícula de dextrán (derecha inferior de la figura 12) al cual esferas más pequeñas que comprenden dextrán/micropartículas de dextrán están unidas y fundidas con las que están unidas y fundidas.

[0047] Así, cuando la proporción del volumen de la primer fase (tal como la fase PEG y sus inclusiones, tal como en un

agente terapéutico) al volumen de la segunda fase (tal como la fase de dextrán y sus inclusiones, tal como las micropartículas de dextrán) es menor de uno entonces la cápsula se forma por autoensamble con el primer núcleo de fase circundado por una segunda concha de fase. Si la composición contiene un agente terapéutico, tal como insulina, que prefiere particionarse en la fase PEG, y las micropartículas de dextrán que prefieren particionarse en la fase de dextrán, entonces el agente terapéutico se particiona selectivamente en el núcleo de PEG mientras que las micropartículas se particionan selectivamente y forman la concha alrededor del núcleo de PEG por autoensamblaje.

[0048] La emulsión se puede preparar al mezclar separadamente el dextrán preparado y fases PEG y ambas pueden ser suspensiones de diferentes tipos de partículas que prefieren estar en la fase PEG o en la fase dextrán respectivamente. El principio es que la partición de las moléculas (tal como macromoléculas, ADN, plásmido, etcétera) en diferentes fases de polímero depende de su estructura de superficie y de la energía interfacial de las partículas en las soluciones de polímero.

[0049] La inyección de los sistemas acuosos de dos fases que contienen micropartículas de dextrán cristalizadas en

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tejidos de animales experimentales reveló la formación de implantes con la estructura de cápsula mostradas en las figuras 13 y 14. El volumen de la fase de dextrán es mayor que el volumen de la fase PEG en el sistema bifase o de dos fases. Ambas figuras 13 y 14 muestran que una cápsula con un núcleo PEG y una micropartícula de dextrán y una concha de dextrán/micropartícula de dextrán formada por autoensamblaje in vivo (es decir, después de inyección en tejido de mamífero). La concha comprende regiones microporosas entre micropartículas adyacentes así, también regiones microporosas en las micropartículas mismas.

[0050] Un ejemplo no limitante de un método para formar la estructura de cápsula proveniente de un sistema de dos fases es como sigue. 10 g de dextrán T40 (peso molecular 40 kDa) y 2 g de PEG son disueltos en 88 ml de solución de insulina (Actrapid®) que contienen 1,000 Ul a los cuales 25 g de las micropartículas de dextrán cristalizadas se agrega. Estas etapas son desarrolladas bajo condiciones de flujo laminar. La mezcla es agitada sobre un agitador magnético a 100 rpm a temperatura ambiente durante 30 minutos para formar una mezcla homogénea (es decir, una suspensión). 1.0 g de suspensión contiene 8 Ul de insulina.

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[0051] Se debe notar que las micropartículas de dextrán se pueden preparar de una solución de dextrán con diferente peso molecular que la solución de dextrán que se suministra en el sistema de dos fases. Así, las micropartículas de dextrán cristalizadas se pueden formar en una solución de dextrán de peso molecular mas bajo, tal como una solución de 2 a 20 kDa, que la solución de dextrán que se suministra en el sistema de dos fases, que puede ser una solución de dextrán de 40 a 500 kDa, tal como una solución de 40 a 75 kDa. Esto es ventajoso porque las soluciones de dextrán de peso molecular mayor tal como soluciones de 40 y 70 kDa, han recibido aprobación regulatoria más antes se pueden utilizar para formar una concha de una cápsula a concentraciones inferiores. Las soluciones de peso molecular mas bajo se pueden utilizar para disminuir el tiempo de cristalización sin la solución de dextrán de peso molecular mas bajo que esta suministrando de hecho in vivo. Adicionalmente, las micropartículas de peso molecular mas bajo pueden disolverse más fácil in vivo.

[0052] La estructura de la cápsula formada de un sistema de dos fases en ventajosa porque esta permite una liberación mas homogénea y prolongada del agente terapéutico proveniente del núcleo que de la composición que comprende una fase simple que contiene las micropartículas. Adicionalmente, se cree que al utilizar la estructura de la cápsula, una

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cantidad inferior de micropartículas se puede necesitar para lograr la misma o mejor liberación temporizada de un agente terapéutico que si se utilizara un sistema de fase simple. Adicionalmente, al controlar la cantidad de micropartículas en el sistema de dos fases, se cree que el grosor de la concha de micropartículas se puede controlar. Una concha mas gruesa da como resultado una mayor cantidad de micropartículas en el sistema de dos fases.

Así, la cantidad, duración y/o temporización de la liberación del agente terapéutico proveniente del núcleo de la cápsula se puede controlar al controlar el grosor de la concha. Por lo tanto, el perfil de liberación del agente terapéutico se puede acondicionar para cada paciente o grupos de pacientes.

[0053] Se debe notar que aunque el PEG y los dextranos son utilizados como ejemplos de los materiales de las dos fases, cualquiera otros materiales adecuados que muestran el siguiente comportamiento de partición pueden ser utilizados en su lugar. La figura 15A ilustra esquemáticamente el comportamiento de partición de diferentes tipos de partículas en un sistema acuoso de dos fases. Por ejemplo, tres tipos de moléculas o agregados de moléculas, que son preferiblemente partículas 10, 12 y 14, y dos fases 16 y 18 son mostradas en

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la figura 15A. Sin embargo, puede haber dos, o más de tres tipos partículas. Las partículas pueden ser micropartículas tales como microesferas y nanoesferas preparadas de materiales orgánicos y/o inorgánicos, liposomas, células vivas, virus y macromoléculas. El primer tipo de partículas 10 preferiblemente se segrega en la primer fase 16. El segundo tipo de partículas 12 se segrega preferiblemente al límite de la primera 16 y segunda fases. El tercer tipo de partículas 14 se segrega preferencialmente en la segunda fase 18. Así, por analogía con el ejemplo no limitante previo, las primeras partículas 10 pueden comprender un agente terapéutico, el segundo 12 y/o las terceras partículas 14 pueden comprender micropartículas de dextrán cristalizado, la primer fase 16 puede comprender una fase PEG y la segunda fase 18 puede comprender una fase de dextrán.

[0054] Si una cantidad más pequeña de la primer fase 16 se suministra en una cantidad mayor de la segunda fase 18, como se muestra en el área 20 de la figura 15A, entonces se forma una estructura tipo cápsula que comprende esferas discretas de la primer fase 16 que contiene una concentración del primer tipo de partículas 10, localizado en una segunda fase 18. El segundo tipo de partículas 12 se puede localizar en la interfaz de las fases 16, 18 y activa como una concha de la

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cápsula. Las partículas 14 son dispersadas en la segunda fase 18 y/o forman una concha de la cápsula.

[0055] Como contraste, si una cantidad más pequeña de la segunda fase 18 se suministra en una cantidad mayor de la primer fase 16, como se muestra en el área 22 de la figura 15A, entonces se forma la estructura tipo cápsula que comprende esferas discretas de las segunda fase 18 que contiene una concentración del tercer tipo de partículas 14, localizado en una primer fase 16. El segundo tipo de partículas 12 se puede localizar en la interfaz de las fases 16, 18 y actúa como una concha de la cápsula. Las partículas 10 están dispersas en la primer fase 16 y/o forman una concha de cápsula. Los dos sistemas de fase 20 y 22 pueden ser utilizados como un implante, al ser inyectados, implantados quirúrgicamente o suministrados oralmente a un mamífero, tal como un animal o humano. Así, la cápsula forma un implante tridimensional estructurado, con el núcleo actuando como un reservorio o deposito para liberación controlada del agente terapéutico a través de la concha. Como contraste, un implante con una distribución homogénea de micropartículas es un implante no estructurado. Se debe notar que la estructura formada por los sistemas de dos fases oralmente suministrados pueden ser generalmente descritos como una suspensión

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estructurada que comprende una fase PEG dispersada y una fase de dextrán continúa.

[0056] Adicionalmente, las partículas (es decir, los agregados moleculares) 10, 12 y 14 se pueden sustituir por un material líquido (por ejemplo aceite) o macromoléculas que se particionan selectivamente en una de las dos fases. Por ejemplo, un agente terapéutico, tal como insulina, se puede particionar en la fase PEG del sistema de dos fases PEG/dextrán. En razón a que la insulina se particiona selectivamente en la fase PEG, a la fase PEG forma una insulina que contiene un núcleo de una estructura de cápsula. Se debe notar que mientras que ciertas partículas son agentes terapéuticos selectivamente particionados, al termino "particionado selectivamente" no necesariamente significa que el 100 por ciento de las partículas o la partición de agente terapéutico o el agente terapéutico se particione en una de las dos fases. Sin embargo, la mayoría de la especie selectivamente particionada, preferiblemente el 80% de las especie particionada, se particiona en una de las dos fases. Por ejemplo, aunque la mayoría de la insulina se particiona en la fase PEG, una porción de la insulina puede permanecer en la fase de dextrán.

[0058] Sin desear estar ligado por una teoría particular, el presente inventor considera que la estructura de núcleo de

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[0057] La figura 15B ilustra una imagen por microscopia electrónica de exploración de una sección transversal de una estructura de implante con base en un sistema de dos fases esquemáticamente ilustrado en la figura 15A. Una composición acuosa de dos fases que comprende una primera fase de dextrán, una segunda fase PEG y micropartículas de dextrán cristalizadas se inyectaron en el gel sefarosa. Esta composición de gel se semeja al tejido de mamífero al detener la difusión de micropartículas de dextrán cristalizado proveniente del lado de la inyección. La imagen de la figura 15B ilustra la formación de una estructura de implante de concha-núcleo o de núcleo de concha. El núcleo comprende regiones 30 y 32 circundadas por una concha 34. La región 30 es un vacío que es llenado con la región de fase PEG antes de cortar el gel con imágenes SEM en sección transversal. La región de PEG vierte fuera el gel cuando el gel es cortado durante el seccionamiento transversal. La región 32 es una porción exterior del núcleo que comprende gotas PEG localizadas en las micropartículas de dextrán cristalizado. La región 34 es la concha que comprende las micropartículas de dextrán cristalizadas que circundan y mantienen en su lugar el núcleo que contiene PEG.

[0058] Sin desear estar ligado por una teoría particular, el presente inventor considera que la estructura de núcleo de

concha mostrada en la figura 15B se forma mediante autoensamblaje como se muestra esquemáticamente en la figura 15C. Aunque la primera 16 y segunda 18 fases, tal como las soluciones acuosas de diferentes polímeros incompatibles, están en un recipiente de almacenamiento adecuado 19,tal como un beaker de vidrio o frasco, una fase 16 surge por encima de la otra fase 18. Cuando la composición de dos fases se inyecta en un material que restringe el flujo libre de las fases 16 y 18, tal como un tejido de mamífero o un material de sustrato, tal como un gel que se semeja al tejido, la composición se autoensambla en la estructura con núcleo de concha. Primero, la fase que esta presente en el volumen más pequeño se forma con las formas aproximadamente esféricas, como se muestra en la porción medida de la figura 15C. Entonces las formas esféricas se unen para formar núcleos aproximadamente esféricos de una fase circundados por conchas de la fase exterior, como se muestra en el fondo de la figura 15C. Aunque los dos ejemplos de sistema de fase de un sistema multifase han sido ilustrados, el sistema multifase puede tener más de dos fases si se desea.

D. Terapia de célula

[0059] Los experimentos conducidos con células de mamíferos han mostrado posibilidades para utilizar la técnica

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ya descrita al cuerpo las células absorbidas por la superficie de los agregados de micropartículas de dextrán cristalizadas (ver figuras 3 y 12) o absorbidas por la misma superficie estando dentro de estructuras macroporosas (ver figura 11) o cápsula (ver figuras 13 y 14).

[0060] En general, un material auto organizante para terapias con base en la organización espontánea de sistemas coloidales puede contener micropartículas de dextrán cristalizadas u otras partículas, tal como PLA, PLGA, PMMA, alginato, células, etcétera, para implantes. Por ejemplo, las figuras 16A-16D ilustran una partición de células HeLa y micropartículas de dextrán cristalizadas en un sistema de dos fases dextrán -PEG. La figura 16A, se hizo un sistema de dos fases. El núcleo que comprende la fase PEG que contiene las células es mostrado en la parte superior de la fotografía y el dextrán/concha de micropartícula de dextrán ("CDM") se muestra en la parte inferior. Las gotas de la fase PEG que se segregan hacia fuera de la concha en el núcleo como se muestra en el límite del núcleo en las regiones de núcleo y concha. La figura 16B muestra la partición de las micropartículas de dextrán en un sistema de dos fases PEG/dextrán. La fase PEG 16 esta en la parte superior y la fase de dextrán 18 que contiene las micropartículas de dextrán esta en la parte inferior. Las figura 18C y 16D

muestran las células HeLa sobre la fase de dextrán que contiene la superficie de micropartículas de dextrán cristalizadas.

[0061] Los implantes pueden ser implantes tipo "reservorios" y pueden utilizados para terapia de reemplazo de célula. Los implantes tipo "Reservorio" pueden mejorar las farmacocinéticas (sin "efectos ráfaga") y aseguran la liberación sostenida de sus fases biológicamente activas. El "núcleo" del implante tipo "reservorio" puede contener cualquier tipo de sustancia o sustancias activas además de la estructura del implante que esta siendo "cargado" con células. El núcleo puede contener péptidos terapéuticos y proteínas, ácidos nucleicos, vacunas, virus y células.

[0062] la figura 17 ilustra esquemáticamente un ejemplo de terapia de de reemplazo de célula con base en material auto organizante para principio de terapias. Por ejemplo, los implantes de poro cerrado basados en micropartícula de dextrán cristalizadas se pueden utilizar para la terapia de diabetes tipo 1, terapia de cáncer, terapia de parkinson y otras terapias adecuadas basadas en el uso de las proteínas y péptidos terapéuticos. En la figura 17, el implante 40 contiene una micropartícula de dextrán cristalizada 12, 14 cápsula o concha que encapsula las células transplantadas 42.

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Se pueden utilizar cualquier célula adecuada, tal como una insulina que produce beta-células, células K, células pancreáticas o células madre. Como se muestra en la figura 17, la cápsula de micropartículas de dextrán cristalizadas es porosa a la glucosa sanguínea 44 (así como también al oxigeno, los nutrientes y otros estímulos), pero son impermeables a las células del sistema inmune que son demasiado grandes para ingresar a la cápsula para atacar las células que producen insulina. La glucosa sanguínea 44 permea a través de la cápsula de micropartículas de dextrán cristalizada y estimula la producción de insulina 46 proveniente de las células que producen insulina. La insulina entonces se difunde a través de la cápsula de micropartículas de dextrán cristalizada hacia la sangre 48.

[0063] Así, las células que producen insulina encapsulada o de micropartículas de dextrán cristalizadas se pueden implantar a un mamífero, tal como un humano, para tratar de diabetes. La cápsula de micropartículas de dextrán cristalizada se puede formar in vivo y no necesita una operación quirúrgica. Tal técnica es conocida en el arte como "Ingeniería de Tejido Inyectabe". Adicionalmente, la cápsula de micropartículas de dextrán cristalizada no requiere el uso de químicos reticulados tóxicos. Los implantes preferiblemente se pueden utilizar para suministrar una

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preparación de insulina de larga acción para suprimir la producción de glucosa hepática y mantener cerca la normoglicemia en el estado de ayuno con un perfil con tiempo de acción sin un pico.

[0064] se debe notar que la terapia de célula se puede utilizar para tratar otras enfermedades. Por ejemplo, las células que producen dopamida encapsulada de micropartículas de dextrán cristalizada se pueden utilizar para tratar enfermedad de Parkinson como se ilustro esquemáticamente en la figura 18. Adicionalmente, como se muestra en la figura 18, la estructura de la cápsula de micropartículas de dextrán cristalizada se puede reversar, de tal forma que las células 42 se forman en la concha que encapsula las micropartículas 12, 14 en el tejido de mamífero 50. Por ejemplo, las células propias del paciente o las células madre se pueden localizar por fuera de las micropartículas. Como contraste, las células provenientes de una fuente diferente del paciente, tal como de un donador, se pueden localizar dentro de la concha de micropartícula como se muestra en la figura 17 para proteger las células de la reacción autoinmune del paciente. Esto abre la posibilidad para ingeniería de tejido in vivo para el tratamiento de condiciones, tal como diabetes y otras.

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E. Sustitutos de injerto de hueso

[0065] Los procedimientos de injerto de hueso involucran el uso de tejido de autoinjerto, en el cual el tejido es cosechado en el paciente, o tejido de aloinjerto, que es tomado de donadores o cadáveres. Los dos criterios básicos son utilizados para juzgar la osteoconducción y la osteoinducción de un injerto exitoso.

[0066] Cosechar el tejido de autoinjerto requiere cirugía en el sitio del donador que pueda dar como resultado sus propias complicaciones, tal como inflamación, infección, y dolor crónico. Las cantidades de tejido de hueso que se pueden cosechar también son limitados, creando un problema de suministro también.

[0067] Los aloinjertos enredan algunos de los inconvenientes de autoinjertos al eliminar la morbilidad del sitio de donador y los temas de suministro limitado. Sin embargo, los aloinjertos presentan también riesgos. Aunque muchos métodos pueden reducir el riesgo de transmisión de enfermedad, el tratamiento suele esterilizar las proteínas y los factores removidos de tejido, reduciendo o eliminando la osteoinductividad del tejido.

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[0068] Adicionalmente, varias alternativas se han desarrollado para autoinjertos y aloinjertos. Muchas de estas alternativas utilizan una variedad de materiales, que incluyen polímeros naturales y sintéticos, cerámicas, y compuesto. Otros métodos alternativos incorporan estrategias basadas en factor y célula que son utilizadas bien sea solas o en combinación con otros materiales.

[0069] Muchos sustitutos de injerto de hueso son naturales o sintéticos se pueden utilizar solos o en combinación con factor de crecimiento recombinante tal como el factor de crecimiento transformante (TGF), factor de crecimiento derivado de plaqueta (PDGF), factor de crecimiento de fibroblasto (FGF) y proteína morfogenética de hueso (BMP). El injerto de hueso basado en célula sustituye el uso de células para generar nuevo tejido solo o son sembrados sobre una matriz de soporte (por ejemplo, células madre mesenquimales).

[0070] Con los sustitutos de injerto de hueso basados en polímero, tanto los polímeros degradables como los no degradables son utilizados solos o en combinación con otros materiales, tal como el polímero de ácido poliláctico de porosidad abierta de Cortoss® (OPLA) de Orthovita, Inc. Los polímeros sintéticos degradables, como los polímeros naturales, son reabsorbidos por el cuerpo. El beneficio de

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tener el implante reabsorbido es que el cuerpo es capaz de sanar completamente por sí mismo sin ningunos cuerpos extraños restantes. Para este fin, las compañías han utilizado polímeros degradables tal como ácido poliláctico y ácido poli-láctico-co-glycólico (PLGA) como dispositivos que permanecen solos y como expendedores para autoinjertos y aloinjertos. Por ejemplo, Bone Tec, Inc, ha desarrollado un matriz de espuma de PLGA porosa al utilizar un proceso direccionado de partícula para inducir porosidad. La OsteoBiologics, Inc, tiene entendedores Immix, un producto PLGA en partícula utilizado como un entendedor de injerto.

[0071] En un aspecto preferido de esta modalidad, las micropartículas de dextrán cristalizadas porosas descritas en la modalidad previa a ser utilizadas como sustitutos de injerto de hueso, en combinación con un agente terapéutico que suministra formación de hueso en sitios especiales de dicho cuerpo. Cualquier agente terapéutico adecuado para formación de hueso se puede utilizar, tal como un péptido, célula madre de o proteína, que incluye pero no esta limitada a las células madre mesenquimales mencionadas anteriormente, TGF, PDGF, FGF y BMP. El agente terapéutico puede estar localizado en los poros de las micropartículas porosas. Por ejemplo, el agente terapéutico se puede suministrar en los

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poros de las micropartículas de dextrán cristalizadas después de cristalización.

[0072] En otro aspecto preferido de esta invención, un método para formación de implante in vivo incluye preparar la suspensión de micropartículas según la primer fase liquida, prepararon una suspensión de micropartículas en una segunda fase liquida inmiscible con la primer fase liquida, preparar una emulsión donde la primer fase liquida, es una fase continua y la segunda fase liquida es una fase dispersada, e inyectar la emulsión en el cuerpo del mamífero. Preferiblemente, la emulsión es utilizada como un sustituto de injerto de hueso.

[0073] Preferiblemente, las micropartículas en la primera y segunda fases liquidas son diferentes. Por ejemplo, las micropartículas en la primer fase pueden ser micropartículas no poliméricas, tal como micropartículas cerámicas porosas, aunque las micropartículas en la segunda fase pueden ser micropartículas de polímero, tal como las micropartículas de dextrán cristalizadas porosas anteriormente mencionadas. Si se desea, la segunda fase liquida puede contener el agente terapéutico que suministra la formación de hueso en sitios especiales del cuerpo. El agente terapéutico puede estar localizado en los poros de las micropartículas porosas.

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F. vehículo de suministro de insulina oral

[0074] En otra modalidad preferida de la presente invención, el presente inventor descubrió que una composición de micropartículas porosas (es decir, microporos) se puede utilizar como un vehículo para el suministro oral de proteínas tal como insulina. Las micropartículas porosas pueden ser micropartículas porosas adecuadas las cuales posibiliten la administración oral de insulina con una reducción significativa en la glucosa de la sangre, tal como una reducción del 30% dentro de los 60 minutos de la administración oral. Preferiblemente, las micropartículas son partículas bioadhesivas, tal como partículas que se adhieren al menos temporalmente a las paredes del intestino del mamífero, para permitirle suministro de insulina a través de la pared del intestino. Más preferiblemente, las micropartículas porosas comprenden las micropartículas de dextrán cristalizada descrita anteriormente.

[0075] En una modalidad preferida de la presente invención mostrada en la figura 19, el presente inventor ha descubierto en una suspensión acuosa de micropartículas de dextrán cristalizadas 12 ,14 e insulina 46 oralmente administradas a los mamíferos 53, tal como los conejos fue aproximadamente

igual de efectiva para reducir los millones de glucosa de la sangre que una inyección intramuscular de insulina sola. La figura 19 ilustra esquemáticamente la insulina 46 que permea a través de las paredes 52 del intestino 53 del mamífero proveniente de la composición oralmente administrada 54 que comprende las micropartículas. En razón a que los conejos son in modulo común para humanos en ensayos de droga, el presente inventor cree que una composición liquida o sólida 54 que comprende micropartículas de dextrán cristalizadas e insulina, tal como una suspensión acuosa, una solución, una tableta o una cápsula, seria también efectiva para reducir los niveles de glucosa de la sangre en seres humanos cuando se administra oralmente.

[0076] Los siguientes ejemplos ilustran el suministro oral de insulina utilizando micropartículas de dextrán cristalizadas. El estudio involucro conejos Chinchilla (2.3±0.2 Kg) y la observación de su respuesta a las suspensiones acuosas oralmente administradas que consisten de micropartículas de dextrán cristalizadas preparadas de acuerdo con el método descrito aquí e insulina recombinante humana.

[0077] 3.0 g de dextrán T20 (Pharmacia, Uppsala, Suecia) se disolvieron en 2.0 g de agua y se ubicaron en una caja a

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temperatura de 60° C. Tres horas más tarde, las micropartículas de dextrán cristalizadas fueron lavadas mediante centrifugación a 3,000 g con 3 X 5.0 ml de agua. Luego las micropartículas de dextrán cristalizadas fueron suspendidas en 2.0 ml de agua y se les permitió secarse a temperatura ambiente. El polvo seco resultante fue utilizado para preparar una insulina que contiene suspensión para el experimento de suministro de insulina oral. Las suspensiones que contienen insulina fueron preparadas al mezclar 250 mg de las micropartículas; 0.3 ml (12 Ul) 0 0.6 ml (24 Ul) de insulina (NovoNordisk Actrapid HM Penfill, 40 UI/ml) y agua destilada para alcanzar un volumen de 2.0 ml.

[0078] Las muestras de la suspensión (2.0 ml) fueron introducidas en las gargantas de los conejos con un catéter seguido por la introducción de 10.0 ml de agua para beber. Los animales no fueron alimentados durante 3 horas antes de la introducción de la suspensión. Las muestras de sangre fueron tomadas de la vena de la oreja del conejo y analizada su concentración de glucosa. La glucosa de la sangre fue medida utilizando el método de glucosa oxidasa sobre un "Analizador de Glucosa con Sistema de Un toque" (Lifescan Johnson &, Milpitas, CA, USA) después de calibración adecuada.

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[0079] Los ejemplos 1 a 8 son ejemplos comparativos que involucran 8 conejos. Los ejemplos 9 a 14 son ejemplos de acuerdo con la presente invención que involucran cinco conejos.

[0080] En los ejemplos comparativos 1 y 2 (experimento de control #1 resumido en la tabla I) se introdujo una solución acuosa de insulina recombinante humana intramuscularmente en dos conejos a una dosis de 12 Ul por animal. En los ejemplos comparativos 3 y 4 (el experimento de control #2 resumidos en la tabla II), los conejos permanecieron intactos (es decir, no les fue suministrada ni insulina ni otra inyección a los dos conejos). En los ejemplo comparativos 5 y 6 (el experimento de control #3 resumidos en la tabla III), una suspensión de las micropartículas de dextrán sin insulina fue suministrada oralmente a los dos conejos. En los ejemplos comparativos 7 y 8 (el experimento de control #4 resumido en la tabla IV), una suspensión de las micropartículas Sephadex G-150 obtenidas comercialmente con insulina (24 Ul) se suministro oralmente a los dos conejos. De acuerdo con el sitio Amersham Biosciences, las partículas de Sephadex® G-150 son micropartículas de gel en forma de glóbulo que tiene un diámetro de 20 a 150 micrómetros, preparadas por dextrán reticulado con epilclorohidrina. En los ejemplos 9-14 de acuerdo a una modalidad preferida de la presente invención

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(resumido en la tabla V), una suspensión de micropartículas de dextrán cristalizadas con insulina (24 Ul) fue suministrada oralmente a cinco conejos. Los resultados son resumidos en las tablas I-V de adelante.

Tabla I

Conejo/ Ejemplo #	Dosis de insulina	0 min Mg/dl	30 min Mg/dl	60 min Mg/dl	90 min Mg/dl	120 min Mg/dl
# 1	12 Ul i.m	91	68	58	49	51
# 2	12 Ul i.m	87	65	64	57	58

Tabla II

Conejo/ Ejemplo #	Dosis de insulina	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	98	90	91	94	87

Tabla III

Conejo/ Ejemplo #	Dosis de insulina	0 min Mg/dl	30 min Mg/dl	60 min Mg/dl	90 min Mg/dl	120 min Mg/dl

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# 5	0.0	92	99	94	95	92
# 6	0.0	90	93	93	93	92

Tabla IV

Conejo/ Ejemplo #	Dosis de insulina	0 min Mg/dl	30 min Mg/dl	60 min Mg/dl	90 min Mg/dl	120 min Mg/dl
# 7	24 Ul por os	85	82	86	81	83
# 8	24 Ul por os	84	75	86	76	77

Tabla V

Conejo/ Ejemplo #	Dosis de insulina	0 min Mg/dl	30 min Mg/dl	60 min Mg/dl	90 min Mg/dl	120 min Mg/dl
# 9	24 Ul por os	94	68	59	58	57
# 10	24 Ul por os	93	64	52	54	52
# 11	24 Ul por os	78	52	51	49	48
# 12	24 Ul por os	92	64	52	53	47

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# 13	24 Ul por os	89	53	48	38	49
# 14	24 Ul por os	97	60	38	54	52

[0081] Los datos en las tablas I-V muestran que la reducción promedio de azúcar (es decir glucosa) en la sangre de los mamíferos es comparable cuando 12 Ul de insulina se administran mediante inyección intramuscular (ejemplos 1-2) y 24 Ul de insulina se administran por os (es decir, oralmente) con micropartículas de dextrán cristalizadas (ejemplo 9-14). En la reducción máxima de glucosa fue aproximadamente 35 a aproximadamente 60 por ciento a los 60 minutos después de la administración oral. El perfil de concentración de glucosa es prácticamente el mismo en los modos de inyección como suministro oral. Se debe notar que fueron administradas otras cantidades de insulina. Por ejemplo, 30 Ul de insulina puede ser administrada. En general, la administración oral de 2 a 3 veces la insulina comparada con la cantidad de insulina inyectada produce una caída similar en el azúcar de la sangre durante hasta las tres horas.

[0082] También conocido el hecho de que la insulina en si misma se degrada por las enzimas del intestino y no se

absorben intacta a través de la mucosa gastrointestinal (Amidon GL, lee HJ, Absorption of peptide and Peptidomimetic drugs, Ann. Rev. Pharmacol. Toxicol. 1994; 34: 321-41). Sin embargo, los ejemplos 9-14 muestran que las micropartículas de dextrán cristalizadas se pueden utilizar como un vehículo para el suministro oral de proteínas, tal como insulina porque el efecto de hipoglucemia recibido fue significativo. Sin desear estar ligado por ninguna teoría o modo particular de acción, el presente inventor considera que el uso de las micropartículas de de dextrán porosas, cristalizadas como una matriz de suministro de insulina en una suspensión acuosa protegieron la insulina de degradación significativa por las enzimas del intestino y le permitieron a la insulina ser absorbidas intactas a través de la mucosa gastro intestinal. La insulina puede estar localizada en microporos en las micropartículas microporosas y/o en los macroporos entre las micropartículas. Como contraste, el uso de micropartículas de dextrán Sephadex G-150 reticuladas con insulina (Tabla IV Ejemplo 7-8) no produjo una reducción apreciable en la concentración de glucosa.

[0083] Como se suministra en los ejemplos 9-14, la concentración de glucosa en la sangre en el mamífero se disminuye al menos 5 por ciento, preferiblemente al menos 30 por ciento, 60 minutos después de la administración de la

composición que contiene las micro partículas de extremo cristalizadas y la insulina al mamífero (es decir el valor de glucosa en la sangre en el mamífero a los 60 minutos después de la administración de la suspensión es al menos 5 por ciento, preferiblemente al menos 30 por ciento inferior que aquella medida precisamente antes de la administración de la suspensión). Preferiblemente, la concentración de glucosa en la sangre en el mamífero es disminuida por al menos 5 por ciento, tal como al menos 30 por ciento, preferiblemente por aproximadamente 35 por ciento a aproximadamente 40 por ciento 30 minutos después de administrar la composición al mamífero. Preferiblemente, la concentración de glucosa en la sangre en el mamífero se disminuye por aproximadamente 35 a aproximadamente 60 por ciento, por ejemplo 35 a 45 por ciento 60 minutos después de administrar la suspensión al mamífero. Más preferiblemente, la concentración de glucosa en la sangre en el mamífero se disminuye durante el periodo completo que había de 30 a 240 minutos, tal como 30 a 120 minutos, después de la administración de la composición al mamífero comparada con la concentración de glucosa en la sangre precisamente antes de la administración. Por ejemplo, la concentración de glucosa en la sangre en el mamífero es preferiblemente disminuida por al menos 10 por ciento, preferiblemente al menos 30 por ciento, más preferiblemente por al menos 35 por ciento, tal como pro 35 a 45 por ciento durante un periodo

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que varía de 30 a 240 minutos, preferiblemente 30 a 120 minutos después de la administración de la composición al mamífero.

[0084] Así, una modalidad preferida de la presente invención suministra un método para disminuir la glucosa de la sangre en un mamífero al administrar oralmente una cantidad terapéuticamente efectiva de una composición comprendida de micro partículas de extremo cristalizadas e insulina. Una cantidad "terapéuticamente efectiva" de las composiciones se puede determinar mediante la prevención o mejoramiento de las condiciones adversas o los síntomas de las enfermedades, daños o desordenes que están siendo tratados, Preferiblemente, la composición comprende una suspensión acuosa de micro partículas de dextrán cristalizadas que tienen un diámetro promedio de aproximadamente 0.5 a aproximadamente 5 micrómetros e insulina. Adicionalmente, las micropartículas son preferiblemente micropartículas porosas que son cristalizadas antes de agregar la insulina a la suspensión, de tal forma que la insulina se localiza en contacto con una superficie de las micropartículas y/o en los poros de las micropartículas.

[085] Las micropartículas cristalizadas preferiblemente están comprendidas de moléculas de dextrán (es decir

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moléculas de polímero).que son mantenidas juntas por una pluralidad de enlaces de hidrógeno, fuerzas de Van Der Waals y/o enlaces iónicos y que no tienen sustancialmente enlaces covalentes entre las moléculas de dextrán. Así, las moléculas en las micropartículas son preferiblemente no intencionalmente reticuladas (es decir no se lleva a cabo una etapa de reticulación) y las micropartículas no contienen enlaces covalentes entre las moléculas o menos del 10 por ciento de enlaces covalentes entre las moléculas.

[0086] Aunque una composición de una fase 54 que comprende insulina en micropartículas se ilustra en la figura 19, una composición de dos fases, descrita anteriormente e ilustrada en las figuras 13, 14, 15A, 18B, 15C y 16B puede ser utilizada. Por ejemplo, una composición de dos fases que comprende una fase de dextrán, una fase PEG, micropartículas de dextrán cristalizadas e insulina puede ser utilizada. In vivo, la composición tiene una estructura de cápsula autoensamblada que comprende una pared o concha que contiene micropartícula de dextrán cristalizada o un núcleo que contiene PEG e insulina.

[0087] Preferiblemente, el mamífero que recibe la administración oral de la insulina comprende un humano necesitado de disminuir la glucosa de la sangre, tal como un

humano que sufre de diabetes. Así, la modalidad preferida de la presente invención suministra un método para tratar diabetes en un humano necesitado del tratamiento al administrar oralmente la suspensión de insulina y las micropartículas de dextrán cristalizado descritas anteriormente.

[0088] Cualquier cantidad terapéuticamente efectiva de insulina se puede administrar al mamífero. La cantidad de insulina puede variar con base en el tipo de mamífero (es decir humano o conejo), el peso del mamífero, la composición de la suspensión, la cantidad de la reducción deseada de la glucosa en la sangre y otros factores. Un ejemplo muy limitante del contenido de insulina en suspensión es aproximadamente 10 Ul a aproximadamente 2500 Ul de insulina recombinante humana por un gramo de suspensión, tal, aproximadamente 12 Ul a aproximadamente 30 UI, tal como 24 Ul de insulina recombinante humana, sin embargo, esta cantidad puede variar según se desee.

[0089] La presente invención no se debe considerar como limitada a las modalidades preferidas descritas anteriormente. Otro material de matriz se puede utilizar para el suministro de insulina oral, tal como partículas microporosas orgánicas o inorgánicas. Preferiblemente, las

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partículas son micropartículas que mejoran la penetración de la insulina a través de la mucosa gastrointestinal y/o que estabilizan la composición. Adicionalmente, aunque la suspensión preferiblemente contiene solamente solvente de agua; una matriz; y una solución o suspensión de insulina; el sistema de suministro puede también contener materiales adicionales. Por ejemplo, la composición puede contener una segunda fase tal como las fases PEG de un sistema de dos fases. Así, otro aspecto preferido de la presente invención incluye un método para disminuir la glucosa de la sangre en un mamífero que comprende administrar oralmente una composición que comprende una cantidad terapéuticamente efectiva de insulina y un material de matriz al mamífero para disminuir la glucosa de la sangre del mamífero en al menos 30% 60 minutos después de administrar la suspensión al mamífero. En otro aspecto preferido de la presente invención, un método para suministrar una suspensión a un mamífero comprendido de administrar oralmente la suspensión acuosa de micropartículas de dextrán cristalizada y una cantidad terapéuticamente efectiva de insulina al mamífero.

[0090] Como se anotó anteriormente, las micropartículas de dextrán cristalizadas utilizadas como un material de matriz para la administración de insulina u otras drogas basadas en proteína pueden ser hechas por cualquier método adecuado (ver

figura 1, por ejemplo). Preferiblemente, las micropartículas son hechas mediante el proceso de cualquiera de las modalidades preferidas descritas aquí. Preferiblemente, pero no necesariamente, las micropartículas son formadas en una solución acuosa sin utilizar un solvente orgánico. Así, en un aspecto preferido de la presente invención, una cantidad terapéuticamente efectiva de insulina y micropartículas de dextrán son combinadas en agua después de que las micropartículas han sido cristalizadas para formar una suspensión acuosa de insulina y micropartículas de dextrán cristalizadas. Las micropartículas se pueden agregar al agua antes, en el mismo momento y/o después de agregar la insulina al agua. Adicionalmente, las micropartículas pueden ser oralmente administradas a un mamífero en el solvente en el cual ellas fueron formadas. Alternativamente, ellas pueden ser removidas del solvente en el cual ellas fueron formadas y ubicadas dentro de agua u otra solución acuosa para administración oral o secadas y suministradas en forma sólida, tal como tableta o cápsula, para administración oral.

[0091] La suspensión acuosa de las micropartículas de dextrán cristalizadas y una cantidad terapéuticamente efectiva de insulina (u otras suspensiones adecuadas de insulina y un material de matriz, tal como una suspensión de insulina y micropartículas microporosas) se suministra

preferiblemente como una composición farmaceutita dosificada que es dosificada para administración oral a un humano. En un aspecto preferido de la presente invención, la composición está localizada en un recipiente en una cantidad dosificada para una administración oral simple a un humano. El recipiente puede comprender cualquier recipiente que puede mantener una suspensión, tal como una botella de plástico o vidrio, un tubo, un gotero, una boquilla rociadora, una bolsita y/o otras vasijas adecuadas. Las vasijas contienen una cantidad de suspensión suficiente para una dosis oral simple de la composición.

[0092] En otro aspecto preferido de la presente invención, la composición está localizada en cualquier vasija adecuada en una cantidad adecuada para las dosis múltiple de administración oral. La vasija contiene una instrucción para la administración de dosis oral a un humano. La instrucción puede ser impresa sobre la vasija, tal como ser impresa directamente a la vasija o sobre una etiqueta unida a la vasija, o incluida dentro de la vasija, tal como ser impresa sobre una lámina de papee incluida dentro del a vasija en una caja de cartón o en un sobre de farmacia. Las instrucciones pueden describir la cantidad de la composición que debe ser tomada con cada dosis, la frecuencia con que debe ser tomada la dosis, como medir la dosis de la composición para

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administración oral y/o cualquier otra instrucción adecuada para droga oral para un practicante de cuidados de salud administrante y/o un paciente necesitado de la droga. Alternativamente, las instrucciones pueden comprender direcciones para acceder electrónica o audiblemente la dosis y las instrucciones de administración, tal como un enlace a una red que contiene las instrucciones o número telefónico o regrabar donde son suministradas las instrucciones audiblemente.

[0093] En otro aspecto preferido de la presente invención, la suspensión acuosa de micropartículas de dextrán cristalizadas y una cantidad terapéuticamente efectiva de insulina localizada en una vasija son suministradas en un kit de composición farmacéutico con instrucciones para administración oral de la composición a un humano necesitados de esto. El kit puede comprender instrucciones impresas sobre el recipiente o sobre una etiqueta adherida a la vasija o una hoja de papel incluida dentro de la vasija, tal como en una caja de cartón o sobre de farmacia que incluye una botella (es decir vasija) y la lámina de instrucciones.

[0094] Se debe notar que la composición para administración oral puede estar en la forma de una suspensión acuosa, pero otras formas de suministro se pueden utilizar

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para disminuir la glucosa de la sangre en un mamífero. Por ejemplo, las micropartículas de dextrán cristalizado porosas y la insulina pueden ser oralmente administradas en la forma de una tableta o una cápsula.

[0095] Para administrar oralmente la composición en forma sólida o un mamífero, tal como un humano, la solución de micropartículas de dextrán cristalizadas e insulina es secada primero, tal como secada por congelamiento, para formar un polvo. El polvo puede ser comprendido en una tableta, junto con excipientes opcionales farmacéuticamente aceptables ubicados en una cápsula farmacéuticamente aceptable.

[0096] En otro aspecto preferido de la presente invención, la composición que comprende micropartículas de dextrán cristalizadas y una cantidad terapéuticamente efectiva de insulina puede ser administrada a un mamífero, tal como un humano por inhalación. En este caso, la composición está ubicada en una vasija adaptada para administrar una composición farmacéutica a un mamífero con inhalación, tal como un inhalador que suministra una dosis medida de una composición cuando se oprime o se presiona. Preferiblemente, la composición se suministra a un mamífero a través de la boca al rociar la composición en forma de solución o suspensión de un inhalador. Preferiblemente, la composición

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se suministra a los pulmones del mamífero (es decir suministro pulmonar).

G Vehículo de Suministro de Insulina inyectable.

[0097] El presente inventor ha descubierto que una composición de micropartículas de dextrán cristalizadas en insulina inyectada a mamíferos, tal como ratas y conejos, inesperadamente extendida la duración de la eficacia de la insulina comparada con las inyecciones de la misma dosis de la misma insulina sola. La figura 20 ilustra esquemáticamente la formación de un implante 40 en un mamífero 53 por la inyección de una composición de una fase que comprende las micropartículas 12, 14 y la insulina 46 que utiliza una jeringa 56. La figura 21 ilustra esquemáticamente la formación de un implante estructurado 40 en un mamífero 53 mediante la invención de una composición de dos fases que comprende una fase de dextrán 18 que contiene particionar selectivamente micropartículas de dextrán cristalizado 12, 14 y una fase de PEG 16 que contiene un agente terapéutico selectivamente particionado 10 que comprende insulina. La fase de dextrán 18 forma una concha alrededor de un núcleo de fase PEG 16. En razón a que las ratas y los conejos son un modelo común para humanos en ensayo de drogas, el presente inventor en que las comprendidas micropartículas de dextrán

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cristalizadas y la insulina serían también efectivas para extender la duración de la eficacia de la insulina cuando se inyectan a adultos y niños humanos.

[0098] Los ejemplos 15-22 ilustran la ventaja de utilizar micropartículas de dextrán cristalizadas como un vehículo de suministro de insulina inyectable comparado con la insulina inyectada sola. El experimento involucró ratones .y la observación se hizo de su respuesta a una suspensión acuosa inyectada subcutáneamente que consiste de micropartículas de dextrán cristalizadas e insulina recombinante humana (NovoNordisk Actrapid HM Penfill®, 40 UI/ml).

[0099] La suspensión fue preparada como sigue. 5.0 g de Dextrán T10 (Pharmacia, Uppsala, Suecia, se disolvió en 20.0 g de agua. La solución se filtró a través de un filtro de 0.22 µm (Millipore, Bedford, MA) y se secó por congelamiento. 3.0 g del polvo resultante se disolvieron en 3.0 g de agua estéril y se ubicaron en una caja a una temperatura de 60° C. 6 horas más tarde, las micropartículas de dextrán cristalizadas fuero lavadas mediante centrifugación a 3000 g con 3 x 5.0 ml de agua estéril. Finalmente, la suspensión de micropartículas de dextrán cristalizadas producidas se mezclaron con solución de insulina acuosa y utilizada en el experimento con ratones. Las muestras de las suspensiones

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fueron introducidas en las patas de los ratones y las muestras de la sangre del animal fueron tomadas de cada una de las colas de los ratones y analizada su concentración de glucosa. La glucosa de la sangre se midió utilizando el método de glucosa oxidasa sobre un analizador de glucosa con sistema de un toque (Lifescan, Johnson, Milpitas, CA, USA) después de la calibración adecuada.

[00100] En el ejemplo comparativo 15, no se inyecto insulina al ratón En los ejemplos comparativos 16, 17 y 21, la insulina sola (0.5 Ul) se inyectó en los tres ratones. Los ejemplos 18-20 y 22, la insulina, 0.5 Ul) y el implante de micropartículas de dextrán cristalizadas se inyectó a los cuatro ratones. Los resultados son resumidos en la Tabla VI.

Tabla IV

Ej #		Glucosa 0min Mmol/l	Glucosa 15 min Mmol/l	Glucosa 30 min Mmol/l	Glucosa 45 min Mmol/l	Glucosa 120 min mmol/l	Glucosa 210 min Mmol/l	Glucosa 270 min Mmol/l	Glucosa 390.min mmol/l
15	Ratón Intacto	7.9	8.1	8.2	8.4	-	-	-	-
16	Insulina 0.5 UI	5.9	3.3	2.7	1.8	0.9	3.5	3.0	3.2
17	Insulina 0.5 UI	8.1	3.8	2.8	1.9	0.9	3.7	3.4	3.5
18	Insulina 0.5 Ul con micropart	6.0	4.3	3.2	2.5	0.8	0.8	0.9	0.7

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	ículas de dextrán cristalizadas								
19	Insulina 0.5 Ul con micropartículas de dextrán cristalizadas	6.9	5.6	4.1	3.4	-	1.2	-	1.6
20	Insulina 0.5 Ul con micropartículas de dextrán cristalizadas	5.9	3.5	2.9	1.9	1.2	1.0	1.0	0.7
21	Insulina 0.5 Ul (promedio)	7	3.6	2.8	1.9	0.9	3.6	3.2	3.4
22	Insulina 0.5 Ul con micropartículas de dextrán cristalizadas (promedio)	6.3	4.5	3.4	2.6	1.0	1.0	1.0	1.0

[0101] La reducción promedio de azúcar en la sangre (es decir glucosa en la sangre) de animales es muy diferente cuando fueron aplicadas 0.5 Ul i.m. con y sin micropartículas de dextrán cristalizadas. Como se muestra en la Tabla IV, el nivel de glucosa de los ratones de los ejemplos comparativos 16, 17 y 21 es aproximadamente igualo menos que el nivel de glucosa en ratones de los ejemplos 18-20 y 22 durante los primeros 45 minutos después de inyección. El nivel de glucosa

es aproximadamente el mismo en ratones de ambos ejemplos comparativos 16, 17 y 21 y ejemplos 18-20 y 22, 120 minutos después de inyección. Sin embargo, el nivel de glucosa en los ratones de los ejemplos comparativos 16, 17 y 21 es aproximadamente 3 veces mayor que el nivel de glucosa en los ratones de los ejemplos 18-20 y 22 de 210 a 390 minutos después de la inyección. De hecho, el nivel de glucosa de la sangre en los ratones en los ejemplos 18-20 y 22 no se incrementa sustancialmente (es decir no se incrementa en más del 10%, permaneciendo lo mismo o disminuido) de 120 minutos a 390 minutos después de la inyección. Como contraste, el nivel de glucosa en la sangre en los ratones en los ejemplos comparativos 16,17 y 21 inyectados con la misma cantidad de insulina no se incrementó sustancialmente desde 120 a 390 minutos después de la inyección. La inyección de micropartículas de dextrán cristalizado/insulina disminuyo la glucosa de la sangre durante un periodo mayor que una inyección de insulina de la misma dosis sola. Así, la composición que contiene micropartículas de dextrán cristalizadas e insulina pueden ser dosificadas para inyección.

[0102] Los siguientes experimentos sobre conejos también demuestran como la inyección de micropartículas de dextrán cristalizadas/insulina disminuye la glucosa de la sangre y

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mantiene un nivel basal de insulina en la sangre durante un periodo de tiempo mayor que una inyección de la misma insulina de la misma dosis sola. Una composición subcutáneamente inyectada que comprende insulina de corta acción Actrapid HM® y micropartículas de dextrán cristalizadas fue encontrada inesperadamente que extiende la duración de la eficacia de esta insulina de corta acción para exceder aquella de la insulina de larga acción inyectada subcutáneamente Monotard HM® sola.

[0103] El término duración de eficacia significa disminución de la concentración de glucosa de la sangre y/o mantener un nivel basal de concentración de insulina en la sangre a niveles deseados independientes de los eventos externos que originan los picos en la glucosa de la sangre, tal como al comer. Así, el término duración de la eficacia es un término relativo que compara la eficacia de la insulina y composición de micropartículas de tal forma que la misma dosis de la misma insulina sola. En otras palabras, la duración de la eficacia es una duración de acción o una duración de efecto farmacológico, que puede ser medido de un paciente en estado de ayuno para comparar la eficacia de insulina y la composición de micropartícula con aquella de la misma dosis de la misma insulina sola.

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[0104] Como se muestra en las figuras 22A y 22B, la composición que comprende insulina de corta acción Actrapid HM® y micropartículas de dextrán cristalizadas prolongo la absorción de insulina y extendió el efecto hipoglisémico (es decir, la duración de la eficacia de la insulina al menos 24 horas, tal como aproximadamente 28 a aproximadamente 31 horas, comparada con aproximadamente 2 a aproximadamente 8 horas para la insulina sola Actrapid HM® (figura 22B) y aproximadamente 17 a aproximadamente 24 horas para la insulina sola "Monotard HM®" figura 22A) Ambas insulinas Actrapid HM® y Monotard HM® son productos de Novo Nordisk y la duración anunciada de eficacia de estas composiciones de insulina en humanos obtenida de información de la compañía son de 8 y 24 horas respectivamente.

[0105] En las figuras 22A y 22B, la línea superior ilustra la línea de control para conejos intactos a los cuales no se administro insulina. El eje Y de la figura 22A y 22B es una escala normalizada relativa de concentración de glucosa en la sangre para las mismas 8 Ul de dosis de insulina. Los datos en las figuras fueron ajustados para mostrarse en una gráfica para cada figura y mostrar los niveles de glucosa en la sangre de animales que luego de inyecciones de insulina.

[0106] Los datos mostrados en las figuras 22A y 22B se obtuvieron como sigue. Conejo Chinchilla (2.3±0.3 Kg) fueron monitoreados para su respuesta a inyecciones de una formulación que consiste de micropartículas de dextrán cristalizadas e insulina de corta acción Actrapid®. Las muestras de las formulaciones fueron subcutáneamente inyectadas a los conejos. La insulina de larga acción Monotard HM® (40 UI/ml) e insulina de corta acción Actrapid HM® fueron subcutáneamente inyectadas en conejos separados sin las micropartículas y utilizadas como controles. Las muestras de sangres de animales fueron tomadas de la vela de la oreja de los conejos y analizada su concentración de glucosa. La concentración de glucosa en la sangre se midió con un analizador de glucosa (One Touch® Lifescan, Johnson & Johnson, Milpitas, CA, USA) después de calibración adecuada.

[0107] En los ejemplos comparativos 23 y 24, a dos conejos intactos no les fue suministrada ninguna insulina. En los ejemplos comparativos 25 y 26 una solución acuosa de larga acción de insulina Monotard HM® se introdujo subcutáneamente a dos conejos en una dosis de 8 UI. En los ejemplos 27-29, una suspensión de micropartículas de dextrán cristalizada con insulina de corta acción Actrapid HM® se introdujo subcutáneamente a tres conejos en una dosis de 8 UI. Los

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resultados de los experimentos fueron resumidos en la tabla VII.

TABLA VII

EX#	Dosis de Insulina	0 horas glucosa mmol/L	0.5 horas glucosa mmol/L	1 hora glucosa mmol/L	1.5 horas glucosa mmol/L	2 horas glucosa mmol/L	2.5 horas glucosa mmol/L	16 horas glucosa mmol/L	24 horas glucosa mmol/L	31 horas glucosa 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	3.2	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] Los ejemplos anteriores 27-29 ilustran que la composición de micropartículas de dextrán cristalizadas con insulina de corta acción Acrapid HM® suministra un efecto prolongado que excede el efecto de la insulina de larga duración Monotard HM® y se cree que es comparable con el efecto de la insulina de larga acción (dosificada una vez diariamente) glargine Lantus® de Aventis (ver www.aventis-us.com/Pls/lantus_TXT.html). En adición, la insulina Lantus no debe ser diluida o mezclada con ninguna otra insulina o

solución. Si la insulina Lantus® se diluye o se mezcla, el perfil farmacocinética/farmacodinámico (por ejemplo el efecto de acción, tiempo para el efecto pico) del Lantus® y/o de la insulina mezclada se puede alterar de una manera no predecible. Como contraste, la composición de las micropartículas de dextrán cristalizadas con insulina no esta así limitada porque ninguna insulina adecuada, tal como la insulina humana, se puede utilizar. En la composición de micropartículas de dextrán cristalizadas en insulina, la proporción de insulina y micropartículas puede variar según se desee. Adicionalmente, ninguna insulina adecuada puede ser utilizada para ajustar una terapia de insulina a un paciente individual. Así, el Actrapid HM® fue utilizado en la composición como un ejemplo ilustrativo de una insulina típica y la composición no esta limitada a esta marca de insulina.

[0109] Como se muestra en los ejemplos 23 a 29, la composición que contiene la micropartículas de dextrán cristalizadas y la insulina es efectiva para mantener una duración de eficacia de la insulina durante al menos 30% más, tal como al menos 100% más, preferiblemente 100 a 400% más que la misma dosis de la misma insulina sin las micropartículas. La micropartícula que contiene la composición de insulina es efectiva para mantener un nivel

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bastante deseado en la sangre y una concentración de glucosa en la sangre durante al menos 30% más, tal como 100% a 400% más, que la misma dosis de la misma insulina sin las micropartículas. Así, la duración de la eficacia de la composición que contiene micropartícula es al menos 24 horas, lo cual le permite ser inyectada solamente una vez diariamente al mamífero, tal como un humano necesitado de esto.

[0110] La composición de micropartícula con insulina de larga duración es más segura que las composiciones de insulina de larga duración de la técnica anterior porque estas pueden lograr la eficacia de larga duración sin utilizar una dosis mayor de insulina como las composiciones de la técnica anterior. Por ejemplo, si una dosis de 8 Ul de insulina de corta acción se ha determinado como médicamente segura para un paciente sin un riesgo significativo de sobredosis, entonces la composición que comprende la misma insulina de corta acción y las micropartícula de dextrán cristalizadas puede suministrar una eficacia de duración de más larga acción con las mismas 8 Ul dosis de insulina de corta acción sin un riesgo significativo de sobredosis, aun si toda la insulina es liberada al paciente de una vez. Adicionalmente, esta composición suministra un ahorro de costos comparado con las composiciones de la técnica anterior

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porque esta extiende la eficacia sin incrementar la cantidad de insulina. Las terapias para diabetes de larga acción de la técnica anterior son hechas con análogos de insulina tales como Lantus® de Aventis. Como contraste, la composición que contiene micropartículas de dextrán cristalizadas preferiblemente contiene insulina recombinante humana cuyo perfil de seguridad esta establecido. Así, esta composición reduce el riesgo de reacción o reacciones adversas y número de inyecciones a diabéticos, mejorando de esta manera la calidad de la vida de los diabéticos.

[0111] La composición inyectable puede comprender un sistema de fase simple que comprende insulina de micropartículas o un sistema de dos fases que forma un PEG y un núcleo de insulina y una concha de dextrán o de micropartícula de dextrán para una mayor duración de eficacia. Adicionalmente, la composición comprende un sistema coloidal de una fase o multifase (es decir, una suspensión o una emulsión) que es relativamente fácil de inyectar al mamífero.

[0112] El siguiente ejemplo ilustra el uso de una composición inyectable de dos fases que comprende una fase de dextrán, una fase PEG, insulina micropartículas de dextrán cristalizadas. Se cree que cuando se inyecta a un mamífero,

esta composición forma un implante tipo reservorio estructurado que tiene una estructura de cápsula tridimensional. La estructura de cápsula, las micropartículas se particionan selectivamente en la fase de dextrán y la insulina se particiona selectivamente en la fase PEG. La fase dextrán que contiene las micropartículas forma una concha alrededor del núcleo que comprende la fase PEG que contiene la insulina. Este implante estructurado permite la liberación controlada del núcleo a través de la concha.

[0113] En el ejemplo comparativo 30, 0.5 Ul de insulina Actrapid HM® (100 UI/ml) es subcutáneamente inyectada a un ratón. En ele ejemplo 31, 0.40 g de micropartículas de dextrán cristalizadas son dispersadas en 0.6 ml de una solución acuosa 20% (P/P) de dextrán que tiene un peso molecular de 70 kDa (farmacia, Suecia) para formar una suspensión. 10 mg de PEG que tienen un peso molecular de 6 kDa (Fluka) se disuelve en 0.1 ml de insulina Actrapid HM® (100 UI/ml) para formar una solución. 0.05 ml de PEG y de solución de insulina se mezclan con 0.15 ml de la micropartícula y la suspensión de dextrán para formar una composición o una mezcla de dos fases. 0.02 ml de la mezcla de dos fases que contiene 0.05 Ul de insulina se inyecta subcutáneamente al ratón. Los resultados son mostrados en la Tabla VIII.

Tabla VIII

Ejemplo #	0 min glucosa mmol/L	15 min glucosa mmol/L	30 min glucosa mmol/L	45 min glucosa mmol/L	60 min glucosa mmol/L	120 min glucosa 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] Como se puede ver en la Tabla VIII, la duración de la eficacia de la composición de dos fases fue mayor que aquella de la insulina sola. Adicionalmente, la composición de dos fases disminuyo la concentración de glucosa en la sangre más gradualmente que la insulina sola. Sin desear estar ligado con ninguna teoría particular, estos efectos se creen que se deben a la duración controlada de la insulina del núcleo de la estructura de la cápsula.

[0115] Adicionalmente, la composición que contiene micropartícula puede ser individualmente ajustada para cada paciente al ajustar la cantidad de insulina y/o micropartículas para permitirle al paciente inyectarse la composición al mismo tiempo cada día (es decir, una vez cada 24 horas, una vez cada 48 horas, etcétera). Así, la duración de la eficacia de la composición es ajustable para paciente. Para un sistema de dos fases, el perfil de liberación de

insulina del núcleo de la cápsula se puede ajustar al controlar la cantidad de micropartículas para controlar el grosor de la concha de la cápsula.

[0116] Aunque el inventor no desea estar ligado con ninguna teoría particular, se cree que el efecto de larga duración de la misma dosis de insulina en los ratones y conejos con micropartículas de dextrán cristalizadas se puede explicar por la difusión de las moléculas de insulina desde el implante basado en micropartículas de dextrán cristalizadas (es decir una liberación autocontrolada de insulina). En razón a que los ratones y los conejos son un modelo común para humanos en ensayos de drogas, los datos mostrados en las tablas anteriores VI a VIII sugieren que el uso de los implantes basados en, micropartículas de dextrán cristalizadas hacen posible desarrollar sistemas de suministro de liberación controlada con características farmacocinéticas y dinámicas mejoradas y que cumplen mejor las necesidades de pacientes con insulina basal, tal como humanos.

E. Materiales

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[0117] El termino "insulina" debe ser interpretado por comprender análogos de insulina, insulina humana extraída natural, insulina humana producida recombinante, insulina extraída de fuentes bovina y/o porcina, insulinas y mezclas de cualquiera de estos productos de insulina porcina y bovina producida recombinante. El término pretende comprender el polipéptido normalmente utilizado en el tratamiento de diabéticos en una forma sustancialmente purificada pero comprende el uso del término en su forma farmacéutica comercialmente disponible, que incluye excipientes adicionales. La insulina es preferiblemente producida recombinante y puede ser deshidratada (completamente secada) o en solución.

[0118] los términos "análogo de insulina", "insulina monomérica" y similares son utilizados intercambiablemente aquí y pretenden comprender cualquier forma de "insulina" como se definió anteriormente, en donde uno o mas de los aminoácidos dentro de la cadena de polipéptidos han sido reemplazado con un aminoácido alternativo y/o en donde uno o mas de los aminoácidos a sido suprimido o en donde uno o mas aminoácidos adicionales ha sido agregado a la cadena de polipéptido o la secuencias de aminoácido, que actúan como insulina en los niveles de glucosa de sangre decrecientes. En general, el termino "análogo de insulina" de las modalidades

preferidas de la presente invención incluyen "análogos lispro de insulina", como se describió en la Patente U.S. número 5,547,929 incorporada aquí como referencia en su totalidad; los análogos de insulina incluyen insulina LysPro y la insulina humalog, y otros "análogos de súper insulina", en donde la capacidad de la insulina a la análoga para afectar los niveles de glucosa de la sangre se mejoran sustancialmente comparada con la insulina convencional así como también los análogos de insulina de hepatoselectiva que son mas activos en el higazo que en el tejido adiposo. Los análogos preferidos son los análogos de insulina monoméricas, que son compuestos similares a insulina utilizados para el mismo propósito general que la insulina, tal como insulina lispro, es decir, compuestos que son administrados para reducir los niveles de glucosa de la sangre.

[0119] El termino "análogo" se refiere a una molécula, que comparte una actividad funcional común con la molécula que es considerada como comparable y típicamente comparte las características estructurales comunes también.

[0120] El termino "recombinante" se refiere a cualquier tipo de terapéutica clonada expresada en células procarióticas o una molécula genéticamente trabajada con ingeniería, o una librería combinatoria de moléculas que

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puede ser adicionalmente procesada en otro estado para formar una segunda librería combinatoria, las moléculas especiales que contiene los grupos protectores que mejoran la seguridad fisicoquímica, farmacológica, y clínica del agente terapéutico.

[0121] Adicionalmente, los agentes terapéuticos descritos aquí no están limitados a la insulina. Ningún otro agente terapéutico puede ser utilizado en conjunto con las micropartículas para suministro oral, suministro de inhalación, suministro de inyección suministro de implante quirúrgico.

[0122] Por ejemplo, un agente terapéutico adecuado puede comprender un péptido, polipéptido, una proteína que varia de 0.05 K Dalton a 150 K Dalton en tamaño molecular. En particular el péptido, polipéptido, o agentes terapéuticos de proteína incluyen ayudas para diabéticos; tal como la insulina anteriormente mencionada y los análogos de insulina; la amilina; el glucagon; los tensoactivos; los péptidos inmunomodulantes y las proteínas tales como citokinas, las quimioquinas, las linfoquinas; el taxol; las interleucinas, tales como interleucinas-1, interleucina-2, e interferones; las eritroproetinas; los trombolíticos y las heparinas; las anti-proteasas, la antitripsinas y el amiloride; la rhDNase;

los antibióticos y otros anti-infecciosos; las hormonas y los factores de crecimiento tales como las hormonas paratiroides, los análogos HL-RH y GnRH; los ácidos nucleicos, el DDAVP; calcitoninas; la ciclosporina, la ribavirina; las enzimas; las heparinas; los factores hematopoyéticos; las ciclosporinas; las vacunas; las inmunoglobulinas; los péptidos vasoactivos; los agentes contrasentido; los oligonucleótidos, y los análogos de nucleótido. Otros agentes terapéuticos adecuados incluyen virus, células, genes y otros agentes que tienen propiedades terapéuticas, tales como otras vacunas adecuadas y agentes terapéuticas moleculares.

[0123] El termino "amilina" incluye amilina humana natural, bovina, porcina, de rata, de conejo, así como también sintética, amilina semisintética o recombinante y análogos de amilina que incluyen pramlintida y otros agonistas de amilina.

[0124] El termino "proteínas inmunomodulantes" incluyen citocinas, quimioquinas, componentes complemento de linfoquinas, hormonas de crecimiento, accesorios a sistema inmune y moléculas de adhesión y sus receptores de especificidad humana y no humana. Ejemplos útiles incluyen GM-CSF, G-CSF, IL-2, IL-12, OX40, OX40L (gp34), limfotactina, CD40, CD40L. ejemplos útiles incluyen interleucinas, por

ejemplo interleucinas 1 a 15; interferones alfa, beta o gamma; factor de necrosis tumoral, factor estimulante de la colonia de granulocito-macrófago (GM-CSF),factor estimulante de la colonia macrófago (M-CSF) factor estimulante de la colonia granulosito (G-CSF), quimioquinas, tal como proteína activante de neutrofilo (NAP); quimioactrayentes de macrófago y factor activantes (MCAF), RANTES, péptidos inflamatorios de macrófago MIP-1a y MPI-1b, componentes de complemento y sus receptores, a una molécula accesible, tal como B7.1, B7.2, ICAM-1,2 o 3 receptores de citoquina. OX40 y el ligando OX40 (gp34) son ejemplos adicionales útiles de proteínas inmunomodulatorias. Las proteínas inmunomodulatorias pueden para varios propósitos ser de especificidad humana o animal no humana y puede estar representados, para los presentes propósitos, como puede ser el caso y puede ser conveniente, por dominios extracelulares y otros fragmentos con la actividad de unión de proteína de ocurrencia natural, y muteinas de esta, y sus proteínas de fusión y otras secuencias de polipéptidos, por ejemplo, con dominios constantes de cadena pesada de inmunoglobulina. Donde las secuencias de nucleótidos que codifican mas de una proteína inmunomodulante son insertados, ellos pueden, por ejemplo, comprender mas de una citoquina o una combinación de citoquinas y moléculas accesorias/de adhesión.

[0125] El termino "interferón" o "IFN" como se utiliza aquí significa la familia de proteína especifica de especies altamente homologa que inhibe la replicación viral y la proliferación celular y modula la respuesta inmune. Los interferones están agrupados en tres clases basados en el origen celular y la antigenicidad, a saber, interferón alfa (leucocitos), interferón beta (fibroblastos) y interferón gamma (células inmunocompetentes). Las formas recombinantes y los análogos de cada grupo han sido desarrollados y están comercialmente disponibles. Los subtipos en cada grupo están basados en características antigénicas/estructurales. Al menos 24 interferón alfa (agrupados en subtipos A a H) que tienen diferentes o distintas secuencias de aminoácido han sido identificados por aislar y secuenciar ADN que codifica estos péptidos. Los términos "alfa-interferón", "alfa interferón", "interferón alfa", "interferón de leucocito humano" Y "IFN" son utilizados intercambiablemente aquí para describir los miembros de este grupo. El interferón de leucocito humano preparado de esta manera contiene una mezcla de interferones de leucocito humano que tienen diferentes secuencias de aminoácido.

[0126] El termino "eritropoietina" se aplica a polipéptidos aislados sintéticos, semisintéticos, recombinantes, naturales, humanos, de mono, u otro animal o

producto de polipéptido aislado microbiológico que tienen parte de toda la información estructural primaria (es decir, la secuencia continua de residuos de aminoácido) y una o más de las propiedades biológicas (por ejemplo propiedades inmunológicas y actividad biológica in vivo e in Vitro) de eritropoyetina de ocurrencia natural, que incluye variantes alélicas de esta. Estos polipéptidos son también únicamente caracterizados por ser el producto de expresión de huésped procariótico o eucariótico (por ejemplo por células bacteriales, de levadura de mamífero en cultivo) de secuencias de ADN exógenos obtenidas mediante clonación genomita o de cADN o por síntesis de gen. Los productos de expresión microbiana en células de vertebrados (por ejemplo mamíferos y aves) se puede caracterizar adicionalmente por la libertad de asociación con proteínas humanas y otros contaminantes que se pueden asociar con eritropoyetina en su ambiente celular de mamífero natural o en sus fluidos extracelulares tal como plasma u orina. Los productos de levadura típicos (por ejemplo Saccaromyces cerevisiae) o células huésped de procariote (por ejemplo E. coli) están libres de asocio con cualquiera proteínas de mamífero. Dependiendo del huésped empleado, los polipéptidos de la invención pueden ser glicosilados con carbohidratos de mamífero u otros eucariotes o pueden ser no glicosilados. Los polipéptidos también pueden incluir un residuo de aminoácido

metionina inicial (la posición -1). Los productos de glicoproteina novedosa de la invención incluyen aquellos que tienen unas conformaciones estructural primaria suficientemente duplicadora de aquella de la eritropoietina de ocurrencia natural para permitir la posesión de uno o mas de las propiedades biológicas de esta y que tiene una composición de carbohidratos promedio que difiere de aquella de la eritropoietina de ocurrencia natural (por ejemplo de humano).

[0127] Los términos "heparina" y "trombolíticos" incluyen factores antitaponamiento tales como heparina, heparina de bajo peso molecular, activador de plasminógeno de tejido (TPA), urokinasa (Abokinasa)y otros factores utilizados para controlar tapones.

[0128] Los términos "anti-proteasas" y "inhibidores de proteasa" son utilizados intercambiablemente y aplican a agentes sintéticos, semisintéticos, recombinantes de ocurrencia natural o de ocurrencia no natural, solubles o inmovilizados reactivos con receptores, o actúan como anticuerpos, de enzimas o ácidos nucleicos. Estos incluyen receptores que modulan una respuesta inmune humoral receptores que modulan la respuesta inmune celular (por ejemplo receptores de cedula T) y receptores que modulan una

respuesta neurológica (por ejemplo, el receptor glutamato, el receptor glicina, el receptor gamma-amino butírico (GABA)). Estos incluyen los receptores de citosina (implicados en artritis, choque séptico, rechazo de transplante, enfermedad autoinmune y enfermedad inflamatorias), y mayor histocompatibilidad (MHC) receptores clase I y clase II asociados con presentar antígeno a receptores de célula T citotóxica y/o receptores de célula T ayudante (implicados en enfermedades autoinmunes) y el receptor de trombina (implicado en coagulación, enfermedad cardiovascular). También están incluidos los anticuerpos cuyos autoantígenos que reconocen autoantígenos, tales como aquellos anticuerpos implicados en desordenes autoinmunes y anticuerpos que reconocen antígenos virales (por ejemplo HIV, virus de herpes simple) y/o microbiales.

[0129] Los términos "hormonas" y "factores del crecimiento" incluyen hormonas de liberación de hormona tal como hormona de crecimiento, hormona tiroides, hormona de liberación de tiroides (TRH), hormona de liberación de gonadotropina (GnRH), hormona leuteininzante, hormona de liberación de liberación leuteininzante (LHRH, que incluye los super agonistas y los antagonistas, tales como leuprolide, deltirelix, gosorelina, nafarelina, danazol, etcétera) provenientes de fuentes naturales, humanas, de

porcino, bovino, ovino, sintéticos, semisintéticas o recombinantes. Estas también incluyen análogos de somatostatina tales como octreotide (sandostatina). Otros agentes en esta categoría de bioterapeuticos incluyen medicamentos para contracción uterina (por ejemplo oxitocina), diuresis (por ejemplo vasopresina), neutropenia (por ejemplo GCSF), medicamentos para desordenes respiratorios (por ejemplo dismutasa superóxido), RDS (por ejemplo tensoactivos, que incluyen opcionalmente apoproteinas), y similares.

[0130] El termino "enzimas" incluye deoxiribonucleasa recombinante tal como ADNsa (Genentech) proteasas (por ejemplo proteasa serina tal como tripsina y trombina), polimerasas (por ejemplo; ARN polimerasas, ADN polimerasas), transcriptasas y kinasas inversa, enzimas implicadas en la artritis, osteoporosis, enfermedades inflamatorias, diabetes, alergias, rechazote transplante de órgano, activación de oncogen (por ejemplo dihidrofolato reductasa), transducción de señal, regulación de autociclo, trascripción, replicación de ADN y reparación.

[0131] El termino "ácidos nucleicos" incluyen cualquier segmento de ADN o ARN que contiene nucleósidos de ocurrencia natural o no natural, otros agentes proteinoides capaces de

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unirse específicamente a otros ácidos nucleicos u oligonucleótidos por vía complementaria de unión de hidrogeno y también son capaces de unirse a ligandos de acido no nucleico.

[0132] El termino "vacunas" se refiere a composiciones terapéuticas para respuestas humoral estimulante e inmune celular, bien sea aisladas o a través de una célula que presenta antígeno, tal como una célula dendrítica activada que es capaz de activar células T para producir una respuesta inmune celular multivalente contra un antígeno seleccionado. La célula que presenta antígeno potente es estimulada al exponer la célula in vitro a un complejo de polipéptido. El complejo de polipéptido puede comprender una proteína de unión celular dendrítica y un antígeno polipéptido, pero preferiblemente, el antígeno polipéptido es un antígeno de tumor específico de tejido o un producto de gen o oncogen. Sin embargo, se aprecia que otros antigenes, tales como los antigenes virales se pueden utilizar en tal combinación para producir respuestas inmunoestimulatorias. En otra modalidad preferida, la proteína de unión celular dendrítica que forma parte del complejo de polipéptido inmunoestimulatorio es GM-CSF. En una modalidad preferida adicional, el antígeno de polipéptido que forma parte del complejo es la fosfatasa de acido prostático de antígeno de tumor especifica. En las

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otras modalidades preferidas, el antígeno de polipéptido puede ser cualquiera de los antígenos de péptido de producto de oncogen. El complejo de polipéptido también puede contener, entre la proteína de unión celular dendrítica y el antígeno de polipéptido, un péptido ligador. El complejo de polipéptido puede comprender una proteína de unión celular dendrítica covalentemente ligada a un antígeno de polipéptido, tal como el complejo polipéptido que es preferiblemente formado de una proteína de unión celular dendrítica, preferiblemente GM-CSF, y un antígeno polipéptido. El antígeno polipéptido es preferiblemente un antígeno tumoral especifico de tejido tal como fosfatasa de acido prostático (PAP), o un producto de oncogen, tal como her2, p21RAS, y p53; sin embargo, otras modalidades, tal como agentes antivirales, también están dentro del alcance de la invención.

[0133] El termino "inmunoglobulina" comprende oligonucleótidos de polipéptido involucrados en mecanismos de defensa de huésped, tal como codificar y decodificar por uno o mas vectores de gen, conjugar varias porciones de unión de acido nucleicos en células de defensa de huésped, o acoplar vectores expresados para ayudar en el tratamiento de sujetos humanos o animales. Los medicamentos incluidos en esta clase

de polipéptidos incluyen IgG, IgE, IgM, IgD, bien sea individualmente o en combinación con otro.

[0134] Otros agentes terapéuticos adecuados incluyen hormona adrenocorticotropica, factor de crecimiento epidérmico, factor de crecimiento derivado de plaqueta (PDGF), prolactina, luliberina, agonistas de hormona de liberación de hormona luteinizante (LHRH), antagonistas LHRH, gastrina, tetragastrina, pentagastrina, urogastrina, secretina, encefalinas, endorfinas, angiotensinas, factor de necrosis tumoral (TNF), factor de crecimiento de nervio (NGF), heparinasa, proteina morfogénica de hueso (BMP), hANP, péptido similar al glucagon (GLP-1), interleucina-11 (IL-11), VEG-F, antígeno de superficie de hepatitis B recombinante (rHBsAg), renina, bradikinina, bacitracinas, polimixinas, colistinas, tirocidina, gramicidinas, y análogos sintéticos, modificaciones y fragmentos farmacológicamente activos de estos, enzimas, citokinas, anticuerpos y vacunas.

[0135] El termino micropartículas de dextrán incluye micropartículas de dextrán no sustituidas y micropartículas de dextrán sustituidas. Por ejemplo, las micropartículas de dextrán sustituidas incluyen dextrán sustituido con un grupo adecuado, tal como el grupo metilo, hasta un grado que no enrede la cristalización de las micropartículas de dextrán,

tal como hasta 3.5 o menos por ciento de ramificación. El diámetro de micropartícula promedio es preferiblemente aproximadamente 0.50 a aproximadamente 5 micrómetros, más preferiblemente aproximadamente 1 a aproximadamente 2 micrómetros.

[0136] Además, mientras micropartículas de dextrán poroso no reticuladas, tal como las micropartículas cristalizadas, son preferiblemente utilizadas con el agente terapéutico, otras micropartículas orgánicas e inorgánicas adecuadas pueden ser utilizadas en su lugar, tal como otras micropartículas de polímero que incluyen polisacáridos, PLA, PLGA, PMMA, poliimidas, poliésteres, acrilatos, archilamidas, vinil acetato y otros materiales poliméricos, partículas de biomaterial tal como alginato y células, o partuelas inorgánicas tales como sílica, lirio o fosfato de calcio. Preferiblemente las micropartículas son biodegradables. Preferiblemente, son utilizadas micropartículas porosas. Más preferiblemente, las micropartículas tienen suficiente porosidad para contener el agente terapéutico dentro de los poros y suministrar una liberación temporizada del agente terapéutico proveniente de los poros. En otras palabras, el agente terapéutico es liberado durante el tiempo desde los poros tal como en mas de 5 minutos, preferiblemente en mas de 30 minutos, más preferiblemente en mas de una hora, tal como

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en varias horas a varios días, en lugar de todo en uno. Así, el material de partícula, el tamaño de poro y el volumen de poro se pueden seleccionar con base en el tipo de agente terapéutico usado, el volumen de agente terapéutico necesitado para suministro, la duración del suministro del agente terapéutico, el ambiente donde la gente terapéutico será suministrado y otros factores.

[0137] Así, en un aspecto preferido de la presente invención, el agente terapéutico esta localizado al menos parcialmente en los poros de las micropartículas porosas. Preferiblemente, el agente terapéutico no esta encapsulado en las micropartícula (es decir, la micropartícula no actúa como una concha con un núcleo de agente terapéutico dentro de la concha) y no esta unido a la superficie de la micropartícula. Sin embargo, si se desea, una porción del agente terapéutico también se puede encapsular en una concha de micropartícula y/o esta unido a la superficie de la micropartícula además de estar localizado en los poros de la micropartícula. La localización del agente terapéutico en los poros suministra una liberación temporizada óptima del agente terapéutico. Como contraste, el agente terapéutico unido a la superficie de la micropartícula es a menudo liberado demasiada rápidamente, aunque el agente terapéutico encapsulado de la micropartícula es a menudo no liberado suficientemente pronto

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y es entonces liberado todo de una en la medida en que la concha de micropartículas se desintegra. En un sistema de dos fases, al menos el 80% del agente terapéutico esta preferiblemente localizado en un núcleo circundado por una pared o concha que comprende las micropartículas.

I. Métodos de elaboración

[0138] Las micropartículas pueden ser formadas por cualquier método adecuado. Preferiblemente, las micropartículas son combinadas con el agente terapéutico después de que son formadas las micropartículas. Así, las micropartículas, tal como las micropartículas de dextrán cristalizadas son formadas por cualquier método adecuado y luego el agente terapéutico las micropartículas son combinadas por cualquier método adecuado. Como contraste, en algunos métodos de la técnica anterior, el agente terapéutico es encapsulado en una concha de micropartícula al suministrar el material precursor de micropartícula y el agente terapéutico en una solución y luego cristalizar o reticular el material precursor, tal como un monómero o material oligómero para encapsular un núcleo de agente terapéutico en una concha de micropartícula.

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[0139] Preferiblemente, el agente terapéutico es suministrado en los poros de las micropartículas porosas después de que se forman las micropartículas. Así, las micropartículas porosas son primero formadas y luego se suministra el agente terapéutico en una solución que contiene las micropartículas para permitir el agente terapéutico pernear hacia los poros de las micropartículas. Por supuesto, algunos de los agente terapéuticos también se pueden unir a la superficie de la micropartícula en este proceso.

[0140] Así, un método para elaborar micropartículas de dextrán cristalizado poroso no reticulado incluye la preparación de una solución de dextrán, tal como una solución de dextrán acuoso, que conduce un proceso de cristalización para formar micropartículas de dextrán poroso cristalizado, y si se desea, aislar micropartículas de dextrán poroso cristalizado de la solución. Un agente terapéutico es entonces permeado hacia los poros de las micropartículas al suministrar el agente terapéutico en la solución de cristalización que contiene las micropartículas o al suministrar las micropartículas aisladas y el agente terapéutico en una segunda solución, tal como una segunda solución acuosa. Por ejemplo, las micropartículas de dextrán cristalizadas se pueden formar en una primera solución acuosa de dextrán de bajo peso molecular, tal como una solución de

dextrán de 2 a 20 kDa. Las micropartículas son entonces removidas de la primera solución y luego ubicadas en una segunda solución acuosa de dextrán que tiene un dextrán de peso molecular mayor, tal como una solución de 40 a 500 kDa, por ejemplo una solución de 40 a 75 kDa. La segunda solución puede comprender un sistema de primera fase o de segunda fase, que es luego combinada con una segunda fase, tal como una fase PEG que contiene un agente terapéutico. Un método similar puede ser utilizado con otras micropartículas porosas, donde un agente terapéutico es entonces perneado en los poros de las micropartículas después de que las micropartículas porosas son formadas por cualquier método de formación de micropartícula adecuado, incluyendo, pero no limitado la cristalización. Los componentes de la composición tal como insulina, micropartículas y uno o más fases acuosas se pueden combinar en cualquier orden adecuado secuencialmente o simultáneamente.

[0141] Preferiblemente, las micropartículas son formadas mediante autoensamble de una solución que no contiene solventes orgánicos y promotores de reacción orgánicos que dejan un residuo orgánico de las micropartículas. Así, por ejemplo, las micropartículas de dextrán son preferiblemente formadas mediante autoensamble de una solución de dextrán acuoso. Sin embargo, si se desea, los solventes orgánicos y/o

los promotores de reacción orgánica también se pueden utilizar. En este caso, las microparticulas se pueden purificar antes del uso subsecuente para remover el residuo orgánico dañino.

[0142] Como se describió anteriormente, la estructura de cápsula que tiene el primer núcleo de fase en la segunda pared o concha de fase se pueden formar in vivo o in vitro de una composición de dos fases. La composición puede ser un polvo seco, tal como secado por congelamiento y almacenado como un polvo o una torta porosa. Cuando la composición esta lista para ser administrada al mamífero, se hidrata y se administra la mamífero oralmente o mediante inyección.

[0143] Preferiblemente, la composición que incluye las micropartículas y el agente terapéutico es un sistema coloidal fluible cuando la composición es dosificada para inyección. Ejemplos de un sistema coloidal fluible incluye emulsiones y suspensiones que pueden ser inyectadas a un mamífero que utiliza una jeringa o aguja de calibre común sin dificultad indebida. Como contraste, algunas composiciones de la técnica anterior incluyen un agente terapéutico en un hidrogel de dextrán o en una matriz de dextrán reticulada. Un hidrogel de dextrán y una matriz de dextrán reticulada no son

composiciones fluibles si no están específicamente preparadas.

[0144] En otro aspecto preferido de la invención, las micropartículas comprenden un micropartículas que son adhesivas a la mucosa de los mamíferos. Preferiblemente, las micropartículas adhesivas son más micropartículas porosas descritas anteriormente. Esto mejora adicionalmente el suministro efectivo del agente terapéutico.

[0145] En otro aspecto preferido de la presente invención, las micropartículas comprenden micropartículas cuya superficie ha sido especialmente modificada para mejorar la adhesión del agente terapéutico a la superficie de la micropartícula para optimizar el suministro del agente terapéutico. La superficie de micropartícula puede contener cualquier modificación adecuada que incrementaría la adhesión del agente terapéutico.

[0146] La descripción anterior de la invención ha sido presentada para propósitos de ilustración y descripción. No se pretende ser exhaustivo o limitar la invención a la forma precisa descrita, y las modificaciones y variaciones son posibles a la luz de las enseñanzas anteriores o se pueden adquirir de la práctica de la invención. Los dibujos y la

descripción fueron escogidas con el fin de explicar los principios de la invención y su aplicación practica. Se pretende que el alcance de la invención sea definido por las reivindicaciones anexas a este, y sus equivalentes.

[0147] Todas las publicaciones y solicitudes de patente y patentes citadas en esta especificación se incorporan aquí en su totalidad como referencia.

REIVINDICACIONES

1. Una composición fluible farmacéutica que comprende componentes biodegradables y biocompatibles para ser suministrados dentro o a un cuerpo de mamífero y adaptado para formar un objeto estructurado biodegradable, biocompatible, tridimensional, la composición comprende un sistema coloidal multifase, fluible y agente o agentes terapéuticos para suministrar efecto o efectos terapéuticos locales o sistémicos en o al dicho cuerpo.

2. Una composición farmacéutica para ser suministrada dentro o al cuerpo del animal, que comprende:

una primer fase;

una segunda fase;

primeras moléculas o agregados moleculares que son adaptados para partición preferencialmente de la primer fase; y

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segundas moléculas o agregados de moléculas que son adaptados para partición preferencialmente de la segunda fase;

en donde las segundas moléculas o los agregados moleculares autoensamblan una pared adyacente a las primeras moléculas o a los agregados moleculares cuando la composición se suministra a dicho cuerpo de mamífero.

3. La composición de la reivindicación 1, en donde el sistema coloidal es un líquido de suspensión o micropartículas que contienen emulsión.

4. La composición de la reivindicación 2, en donde:

las primeras moléculas o los agregados moleculares comprenden un agente terapéutico;

las segundas moléculas o los agregados moleculares comprenden micropartículas;

la pared comprende una concha de micropartícula alrededor de un núcleo que contiene agente terapéutico.

5. La composición de las reivindicaciones 3 o 4, en donde las micropartículas son micropartículas porosas y en donde la composición suministra mediante inyección hacia el cuerpo del mamífero.

6. La composición de la reivindicación 5, en donde las micropartículas porosas son micropartículas de polímero.

7. La composición de la reivindicación 6, en donde las micropartículas porosas son micropartículas de dextrán cristalizadas que tienen un diámetro promedio que varía de 0.50 a 5.0 micrómetros.

8. La composición de cualquiera de las reivindicaciones 1 a 7, en donde la composición es un sistema de dos fases líquido que contiene una mezcla de micropartículas.

9. La composición de la reivindicación 8, en donde el sistema de dos fases forma una estructura de cápsula cuando se suministra in vivo, la estructura de cápsula comprende un núcleo de primera fase y una concha de segunda fase que circunda el núcleo.

10. La composición de la reivindicación 9, en donde el agente terapéutico se particiona selectivamente en la fase liquida de núcleo y las micropartículas se particionan selectivamente en la fase liquida de concha.

11. La composición de cualquiera de las reivindicaciones 8 a 10, en donde el agente terapéutico se selecciona de un grupo que consiste de u péptido, una proteína, un acido nucleico, un virus, una célula o una combinación de estos.

12. La composición de cualquiera de las reivindicaciones 9 a 11, en donde el núcleo comprende una fase acuosa PEG y insulina selectivamente particionada y la concha comprende una fase acuosa de dextrán y selectivamente micropartículas de dextrán particionadas cristalizadas.

13. La composición de la reivindicación 2, en donde:

la composición comprende una composición fluible a una composición seca que se puede hacer fluible mediante hidratación,

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las primeras moléculas o agregados moleculares son seleccionados del grupo que consiste de micropartículas, macromoléculas, células, liposomas, ADN, plasmados y proteínas; y

las segundas moléculas o agregados moleculares son seleccionados de un grupo que consiste en micropartículas, macromoléculas, células, liposomas, ADN, plásmido y proteínas.

14. Un método de formación in vivo de un implante biocompatible que comprende:

formar una composición fluible farmacéutica que comprende un sistema coloidal multifase y un agente terapéutico;

inyectar la composición fluible farmacéutica en un cuerpo de mamífero; y

formar un implante biodegradable y biocompatible estructurado mediante autoensamblaje en el cuerpo del mamífero.

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15. Un método para suministrar una composición farmacéutica dentro o hacia un cuerpo de mamífero, que comprende:

una primera fase;

una segunda fase;

primeras moléculas o agregados moleculares que se han adaptado para particionarse preferencialmente de la primer fase; y

segundas moléculas o agregados moleculares que están adaptados para particionarse preferencialmente en la segunda fase; y

suministrar la composición farmacéutica en o hacia el cuerpo del mamífero en donde la segundas moléculas o los agregados moleculares se autoensamblan a una pared adyacente de las primeras moléculas o agregados moleculares cuando la composición se suministra en o hacia dicho cuerpo de mamífero.

16. El método de la reivindicación 14, en donde el sistema coloidal es una suspensión liquida o una emulsión que contiene micropartículas.

17. El método de la reivindicación 15, en donde:

las primeras moléculas o los agregados moleculares comprenden una agente terapéutico;

las segundas moléculas o los agregados moleculares comprenden micropartículas;

la pared comprende una concha de micropartículas alrededor de un núcleo que contiene agente terapéutico.

18. El método de las reivindicaciones 16 o 17, en donde las micropartículas son micropartículas de polímero y el cuerpo del mamífero comprende un cuerpo de humano o un animal.

19. El método de la reivindicación 18, en donde las micropartículas de polímeros son micropartículas porosas.

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20. El método de la reivindicación 19, en donde las micropartículas porosa son micropartículas de dextrán cristalizadas que tienen un diámetro promedio que varían desde 0.50 a 5.0 micrómetros.

21. El método de cualquiera de las reivindicaciones 14 a 20, en donde la composición es un sistema de dos fases que contiene micropartículas.

22. El método de la reivindicación 21, en donde el sistema de dos fases forma una estructura de cápsula cuando se suministra in vivo, la estructura de cápsula comprende un núcleo de primer fase y una concha de segunda fase que circunda el núcleo.

23. El método de la reivindicación 22, en donde el agente terapéutico es particionado selectivamente en el núcleo y las micropartículas son particionadas selectivamente en la concha.

24. El método de cualquiera de las reivindicaciones 21 a 23, en donde el agente terapéutico es seleccionado de un grupo que consiste de un péptido, una proteína, un acido nucleico, un virus, y una célula.

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25. El método de cualquiera de las reivindicaciones 22 a 24, en donde el núcleo comprende una fase acuosa PEG y insulina selectivamente particionada y la concha comprende una fase acuosa de dextrán y micropartículas de dextrán selectivamente particionadas cristalizadas.

26. El método de la reivindicación 15, en donde:

la composición comprende una composición fluible a una composición seca que se hace fluible mediante hidratación antes de ser suministrada al cuerpo del mamífero;

la primeras moléculas o agregados moleculares son seleccionados de un agrupo que consiste en micropartículas, macromoléculas, células, liposomas, ADN, plasmados y proteínas; y

la segundas moléculas o agregados moleculares son seleccionados del grupo que consiste de micropartículas, macromoléculas, células, liposomas, ADN, plasmados y proteínas.

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27. Una composición fluible, que comprende una suspensión coloidal a una emulsión de micropartículas biocompatibles y biodegradables y una etiqueta en un fluido.

28. La composición de la reivindicación 27, en donde:

las micropartículas comprenden micropartículas de dextrán cristalizadas;

el marcador comprende una macromolécula fluorescente que se libera controlablemente de la composición cuando esta se localiza en un cuerpo de mamífero.

29. La composición de la reivindicación 27, en donde la suspensión coloidal comprende una cápsula que tiene una concha de primer fase que comprende un marcador selectivamente particionado y un núcleo de segunda fase que comprende micropartículas selectivamente particionadas.

30. Un método para formación in vivo de un implante biocompatible, que comprende:

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suministrar una composición que comprende una suspensión coloidal o una emulsión de micropartículas biocompatible y biodegradables en un fluido; y

introducir una composición farmacéutica en un cuerpo de mamífero.

31. El método de la reivindicación 30, en donde:

las micropartículas comprenden micropartículas de dextrán cristalizadas;

las micropartículas de dextrán cristalizadas son biocompatibles con el cuerpo del mamífero son biodegradables dentro del cuerpo del mamífero; y

el marcador comprende una macromolécula fluorescente.

32. El método de la reivindicación 30, en donde la suspensión coloidal se autoensambla en una cápsula que tiene un núcleo de primera fase que comprende un marcador selectivamente particionado y una concha de segunda fase que comprende micropartículas selectivamente particionadas cuando la suspensión se introduce en el cuerpo del mamífero.

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33. Un método de terapia celular que comprende suministrar células incluidas o puestas en contacto con una superficie exterior de una cápsula de micropartículas de dextrán cristalizadas en un mamífero necesitado de esto.

34. El método de la reivindicación 33, en donde:

la células están incluidas en la cápsula de tal forma que las células comprenden un núcleo de la cápsula y las micropartículas comprenden una concha que incluye el núcleo; y

la concha es porosa al oxigeno y a los nutrientes pero impermeable a las células del sistema inmune.

35. El método de la reivindicación 34, en donde la células comprenden células que producen insulina que generan y liberan insulina a través de la cápsula hacia la sangre de un mamífero para disminuir la glucosa de la sangre en respuesta a la permeación de la glucosa de la sangre dentro de la cápsula.

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36. el método de la reivindicación 34, en donde las células comprenden células que no son del mamífero al cual la composición esta siendo administrada.

37. El método de la reivindicación 33, en donde:

las células ponen en contacto la superficie exterior de la cápsula; y

las células comprenden las células provenientes del mamífero al cual la composición esta siendo administrada.

38. Un sustituto de injerto de hueso que comprende micropartículas de dextrán cristalizadas.

39. Un sustituto de injerto de hueso de la reivindicación 38, que comprende además un agente terapéutico localizado en poros de las micropartículas que suministran formación de hueso en sitios especiales de un cuerpo.

40. Un método para formar un sustituto de injerto de hueso, que comprende suministrar micropartículas de dextrán cristalizadas porosas un sitio de injerto de

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194

hueso en un cuerpo de mamífero y formar un sustituto de injerto de hueso que comprende las micropartículas de dextrán cristalizadas porosas.

41. El método de la reivindicación 40, que comprende además:

preparar una suspensión den las micropartículas de dextrán cristalizadas porosas en una primer fase liquida;

preparar una suspensión de segundas micropartículas en una segunda fase liquida inmiscible con la primer fase liquida;

preparar una emulsión donde la segunda fase liquida es una fase continua y la primer fase liquida es una fase dispersa; y

inyectar la emulsión al cuerpo del mamífero.

42. El método de la reivindicación 41, en donde:

las segundas micropartículas comprenden micropartículas cerámicas; y

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un agente terapéutica que suministra formación de hueso en sitios especiales del cuerpo es localizado en poros de las micropartículas de dextrán cristalizadas porosas.

43. El método, que comprende:

suministrar micropartículas de dextrán cristalizadas; y

combinar una cantidad terapéuticamente efectiva de insulina y las micropartículas de dextrán cristalizadas en una solución después de que las micropartículas han sido cristalizadas para formar un suspensión de insulina y micropartículas de dextrán cristalizadas.

44. El método de la reivindicación 43, que comprende además administrar la suspensión a un animal.

45. El método de la reivindicación 44, en donde:

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la micropartículas de dextrán cristalizadas comprenden micropartículas que tienen un diámetro promedio de 0.5 a 5 micrómetros;

la etapa de suministrar las micropartículas comprende formar las micropartículas en un solvente no orgánico;

la solución comprende una solución acuosa; y

el mamífero comprende un humano.

46. El método de la reivindicación 44, que comprende además secar la suspensión para suministrar la composición que comprende las micropartículas de dextrán cristalizadas y la insulina.

47. el método para elaborar micropartículas de dextrán poroso cristalizado no reticulado, que comprende:

(a) preparar una solución de dextrán acuoso a la que le falta un solvente orgánico;
(b) conducir un proceso de cristalización para formar las micropartículas de dextrán a una temperatura por encima de la temperatura ambiente de cuarto; y

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(c) aislar las micropartículas de dextrán cristalizado no reticulado de la solución.

48. El método de la reivindicación 47, en donde:

la solución de dextrán comprende dextrán que tiene un peso molecular de 2 a 200 kDa; y

el proceso de cristalización es conducido a una temperatura de 40 a 99°C.

49. El método de la reivindicación 48, en donde:

las micropartículas son espontáneamente formadas de la solución;

la solución de dextrán comprende dextrán que tiene un peso molecular de 20 a 75 kDa; y

el proceso de cristalización es conducido a la temperatura de 40 a 70°C

50. El método de la reivindicación 47, que comprende además combinar las micropartículas con insulina.

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51. un método para elaborar micropartículas de dextrán cristalizado poroso que contienen un agente terapéutico en poros de micropartícula, que comprende:

suministrar una suspensión que comprende micropartículas de dextrán cristalizadas porosas formadas; y

suministrar el agente terapéutico a la suspensión de tal forma que el agente terapéutico se permea hacia los poros de las micropartículas porosas.

52. El método de la reivindicación 51, en donde la etapa de suministrar una suspensión comprende:

preparar una solución de dextrán acuoso;

conducir un proceso de cristalización para formar micropartículas de dextrán cristalizado poroso;

aislar las micropartículas de la solución; y

suministrar las micropartículas en un sistema coloidal que comprende el agente terapéutico.

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53. El método de la reivindicación 52, en donde:

el agente terapéutico comprende insulina; y

el sistema coloidal comprende una suspensión que comprende insulina y las micropartículas.

54. Una composición, que comprende:

insulina; y

micropartículas de dextrán cristalizada poroso;

en donde las micropartículas están formadas antes de la combinación de la insulina y las micropartículas en la composición.

55. la composición de la reivindicación 54, en donde la composición comprende una composición coloidal fluible y las micropartículas comprenden micropartículas que tienen un diámetro promedio de 0.5 a 5 micrómetros.

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200

56. La composición de la reivindicación 54, en donde la insulina esta localizada en poros de las micropartículas de dextrán cristalizado pero no están encapsuladas dentro de cada micropartícula.

57. La composición de la reivindicación 54, en donde la insulina selectivamente particionada en una primer fase de polímero y las micropartículas son selectivamente particionadas en una segunda fase de polímero, de tal forma que la composición forma un implante estructurado luego de la introducción en el cuerpo del mamífero.

58. Una composición, que comprende:

insulina;

un primer polímero;

un segundo poliedro que es compatible con el primer polímero; y

micropartículas.

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261

59. La composición de la reivindicación 58, en donde la composición comprende una composición coloidal fluible y las micropartículas comprenden micropartículas de dextrán cristalizado que tienen un diámetro promedio de 0.5 a 5 micrómetros.

60. La composición de la reivindicación 59, en donde el primer polímero comprende un dextrán y el segundo polímero comprende PEG.

61. La composición de la reivindicación 60, en donde la insulina selectivamente particionada en una fase PEG de micropartículas son selectivamente particionadas en un fase de dextrán, de tal forma que la composición forma un implante estructurado que comprende un núcleo de fase PEG y una concha de fase de dextrán luego de la introducción en un cuerpo de mamífero.

62. Un método para disminuir la glucosa de la sangre en un mamífero, que comprende suministrar una cantidad terapéuticamente efectiva de una composición que comprende micropartículas de dextrán cristalizado e insulina para disminuir la glucosa de la sangre del mamífero, en donde las micropartículas son formadas

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antes de la combinación de la insulina y las micropartículas en la composición.

63. El método de la reivindicación 62, en donde la composición comprende una composición coloidal fluible de las micropartículas comprenden micropartículas de dextrán cristalizadas que tienen un diámetro promedio de 0.5 a 5 micrómetros.

64. El método de la reivindicación 63 en donde:

la composición comprende una composición de dos fases que comprende una fase de dextrán y una fase PEG;

la insulina selectivamente particionada en la fase PEG y las micropartículas son selectivamente partcionadas en la fase de dextrán; y

la composición forma un implante estructurado que comprende un núcleo de fase PEG y una concha de fase de dextrán después de la introducción en un cuerpo de mamífero.

65. El método de la reivindicación 64, comprende además controlar un grosor de la concha con base en el cuerpo

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del mamífero que recibe la composición para controlar la liberación de insulina desde el implante.

66. El método de la reivindicación 62, en donde la composición se suministra a un humano que sufre de diabetes para disminuir la concentración de glucosa de la sangre en el humano.

67. Un inhalador, que comprende:

una vasija adaptada para administrar una dosis de composición farmacéutica a un mamífero mediante inhalación; y

una composición farmacéutica que comprende micropartículas de dextrán cristalizadas y una cantidad terapéuticamente efectiva de insulina localizada en la vasija.

68. Un método para disminuir la glucosa de la sangre en un mamífero, que comprende administrar mediante inhalación una cantidad terapéuticamente efectiva de una composición que comprende micropartículas de dextrán cristalizadas e insulina al mamífero para disminuir la glucosa de la sangre del mamífero.

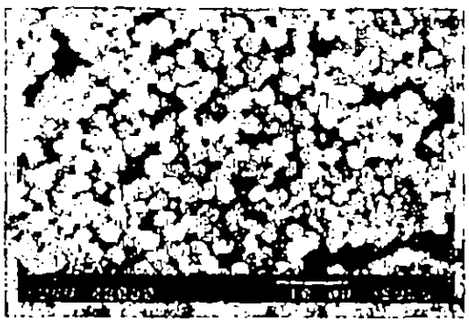


FIG. 1

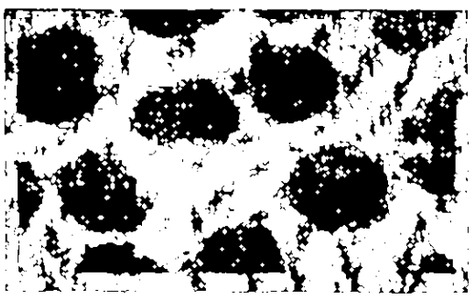


FIG. 2A

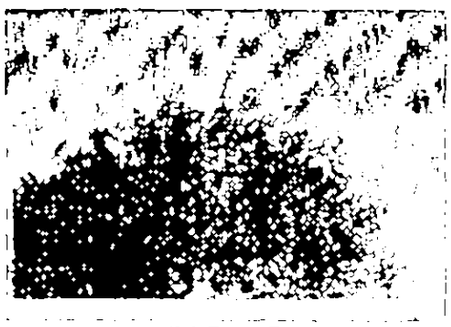


FIG. 2B

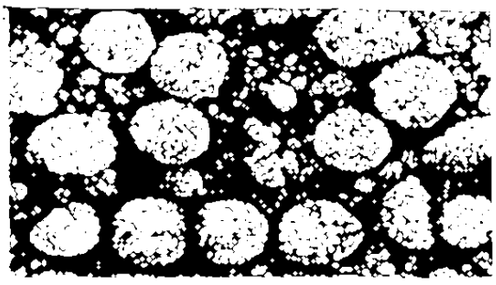


FIG. 3

FIG. 4

FIG. 5

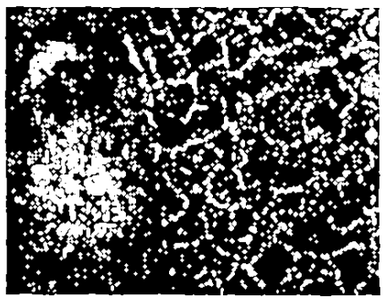


FIG. 6A

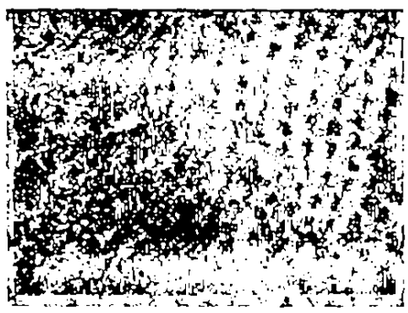


FIG. 6B

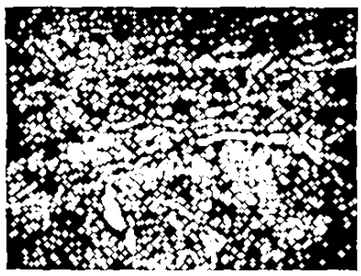


FIG. 6C

FIG. 7A

FIG. 7B

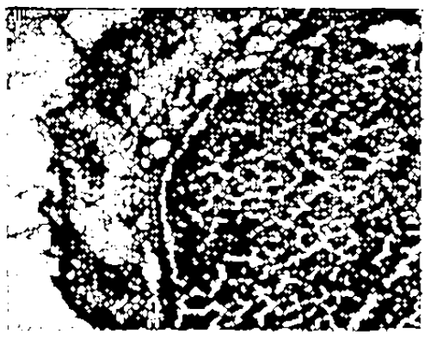


FIG. 8A

FIG. 8B

FIG. 8C

FIG. 8D

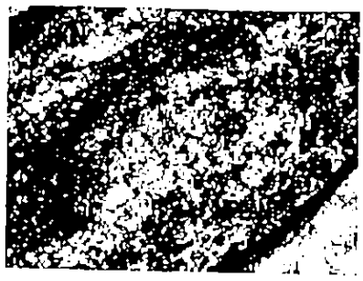


FIG. 8E

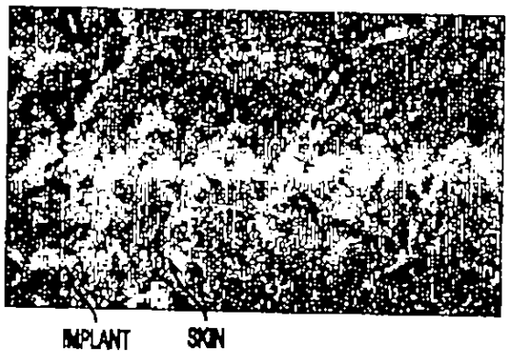


FIG. 8F

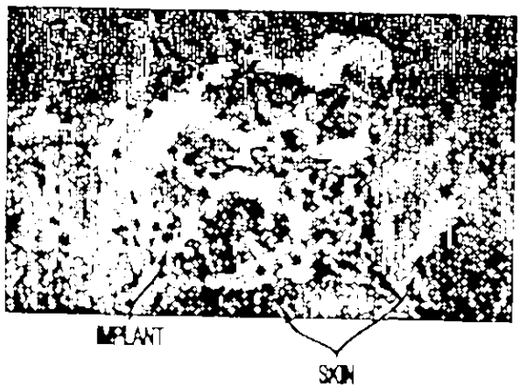


FIG. 8G

FIG. 9

FIG. 10

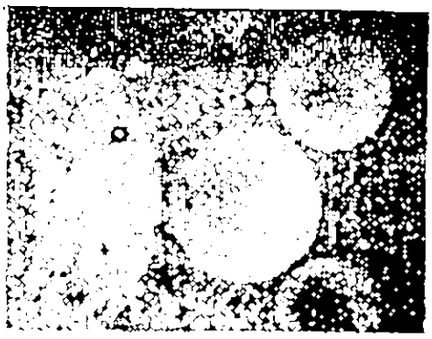


FIG. 11

FIG. 12

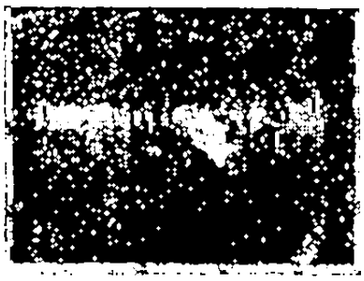


FIG. 13

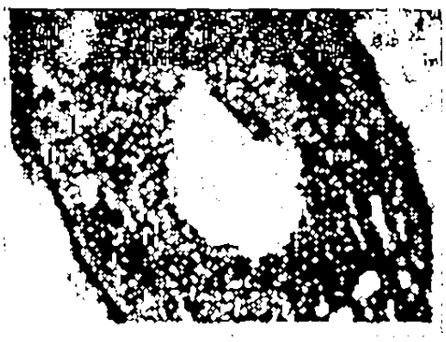
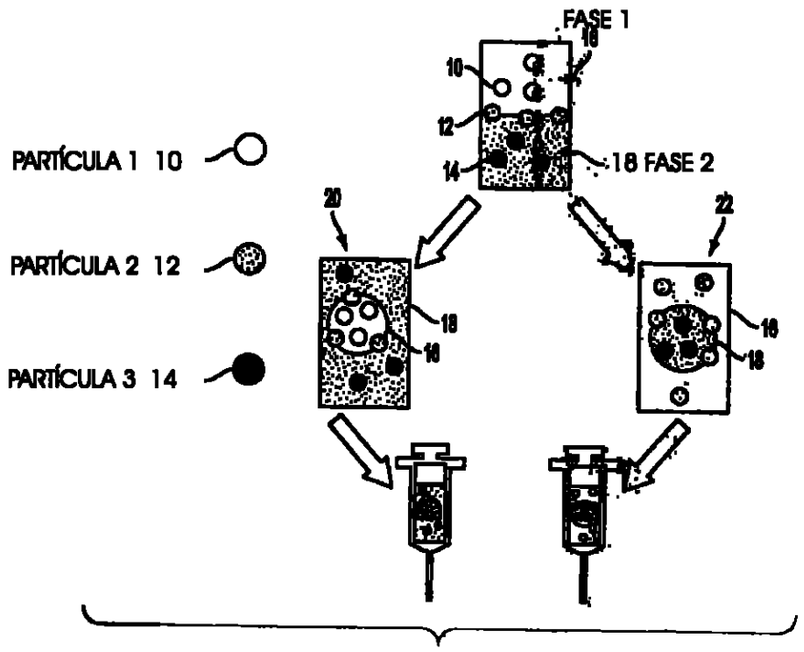


FIG. 14


FASE 1
16
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12
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18 FASE 2
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PARTÍCULA 1 10
PARTÍCULA 2 12
PARTÍCULA 3 14
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FIG. 15A

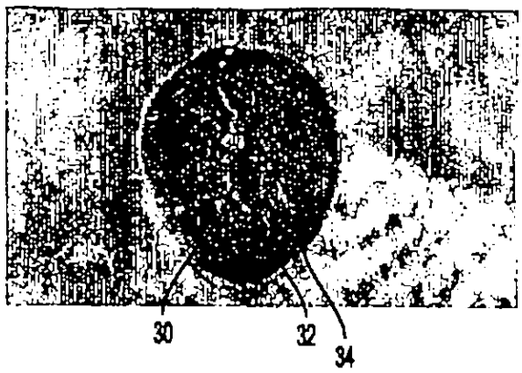


FIG. 15B

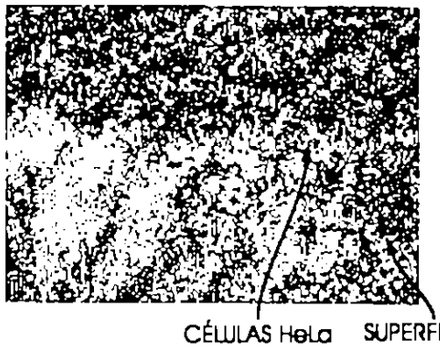


FIG. 16A

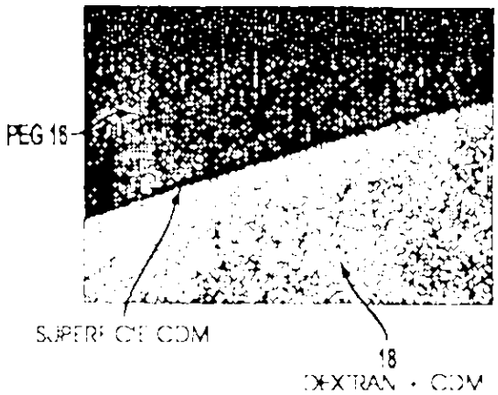
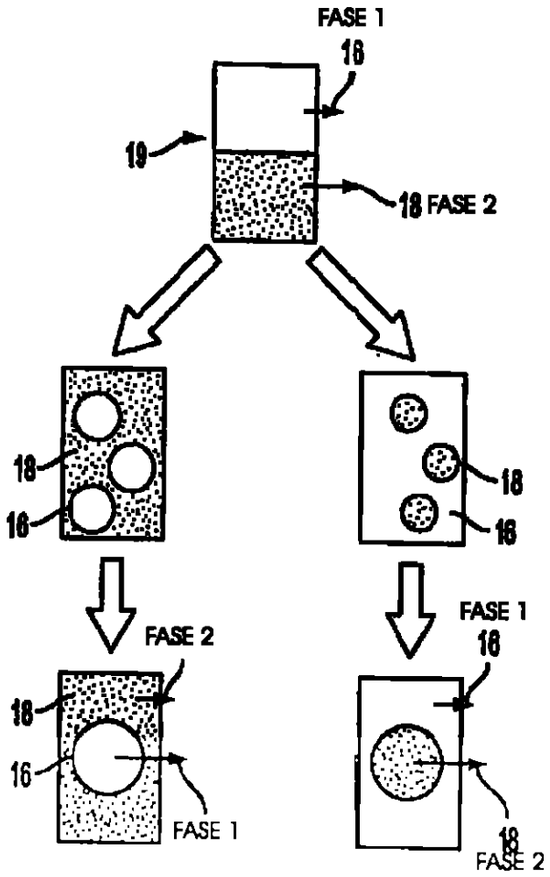


FIG. 16B


FASE 1
16
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FASE 2
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FASE 1
FASE 1
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FASE 2

FIG. 15C

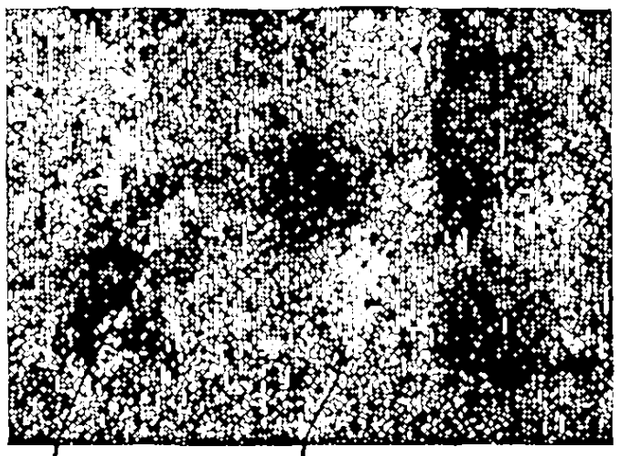

FIG. 16C

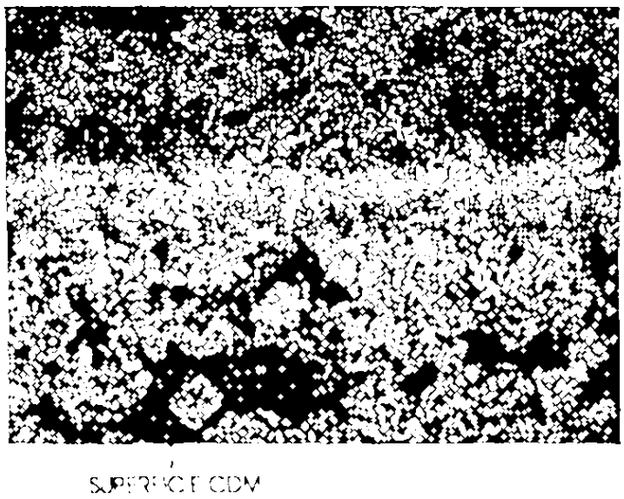

FIG. 16D

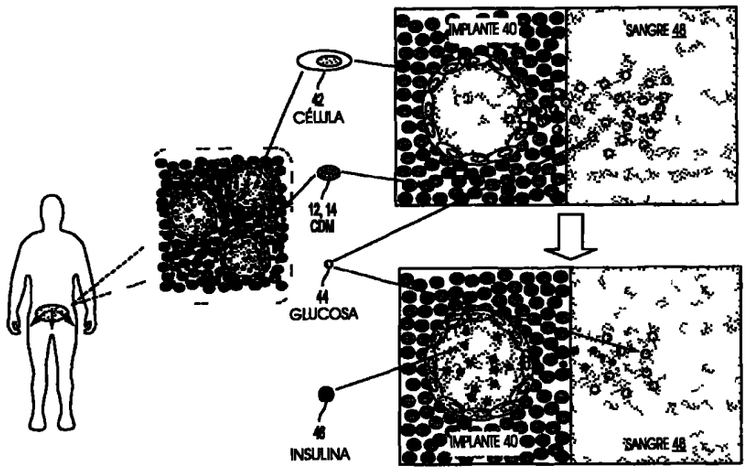
IMPLANTE 40
SANGRE 48
42
CÉLULA
12, 14
CDM
44
GLUCOSA
46
INSULINA
IMPLANTE 40
SANGRE 48

FIG 17

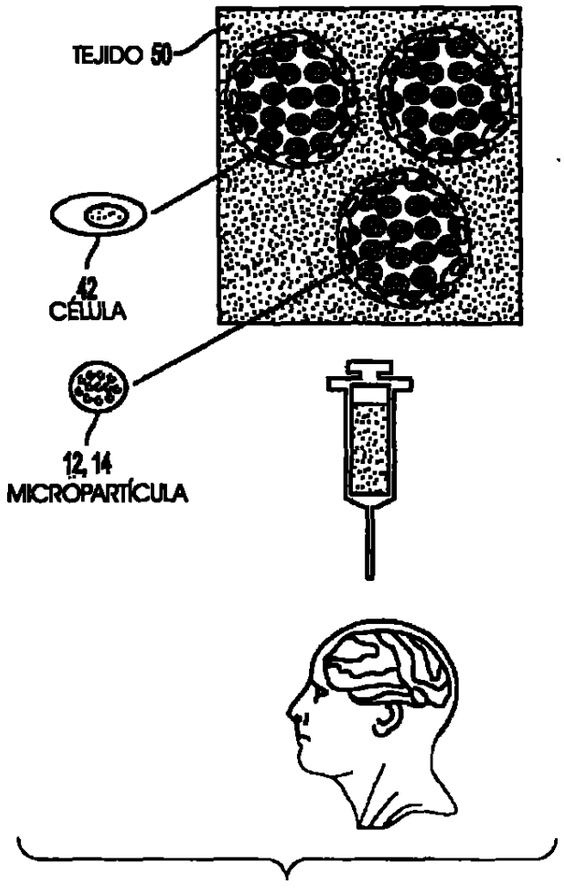

FIG. 18

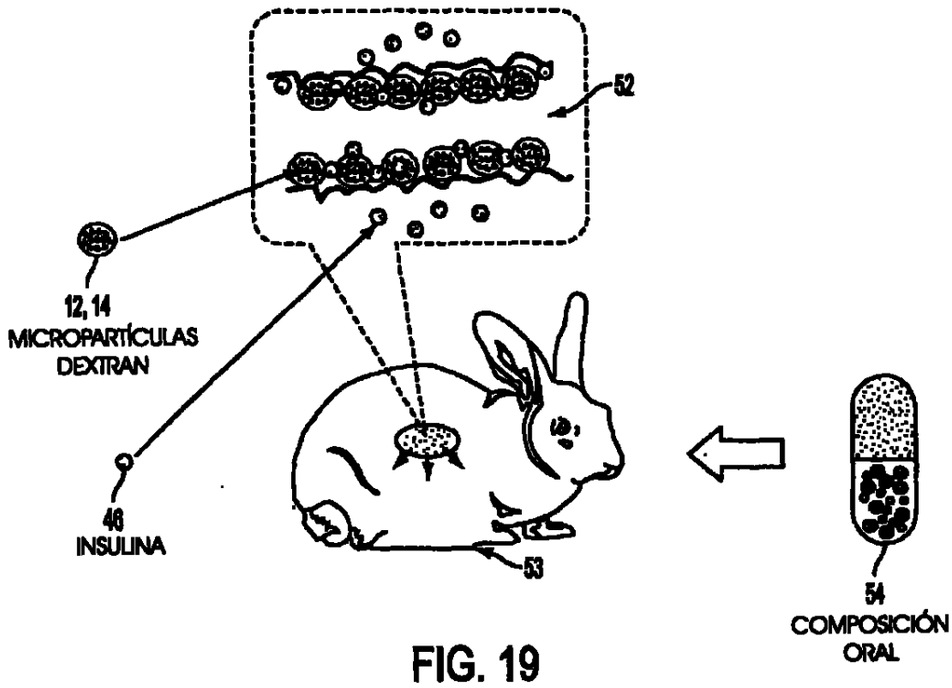
52
12, 14
MICROPARTÍCULAS
DEXTRAN
46
INSULINA
53
54
COMPOSICIÓN
ORAL

FIG. 19

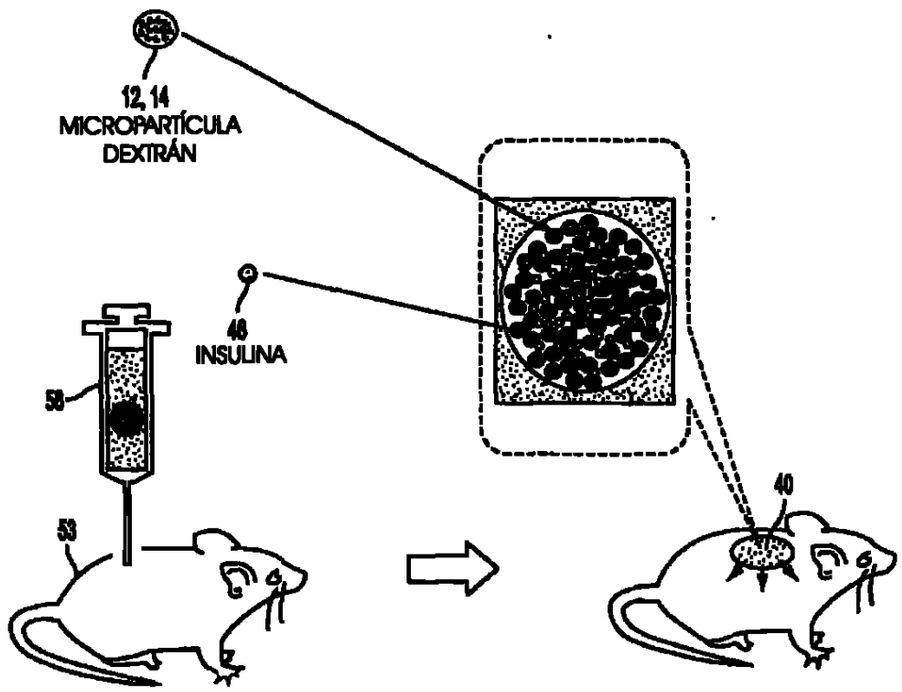

FIG. 20

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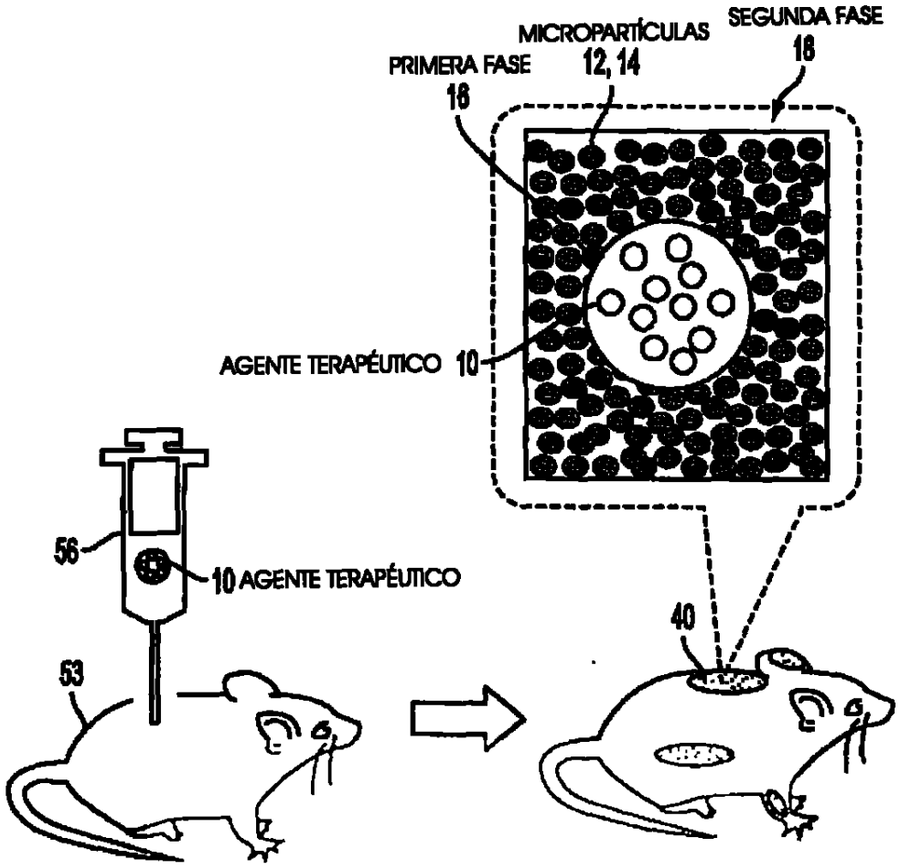
MICROPARTÍCULAS
12, 14
SEGUNDA FASE
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PRIMERA FASE
18
AGENTE TERAPÉUTICO 10
56
10 AGENTE TERAPÉUTICO
53
40

FIG. 21

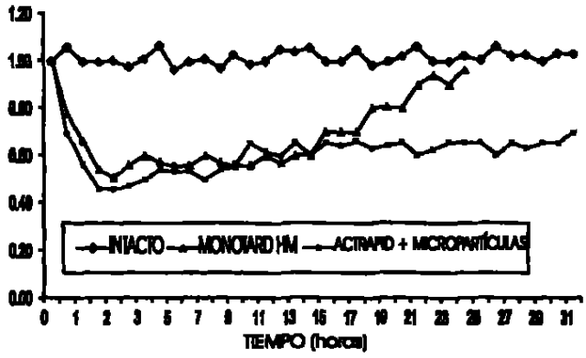

FIG. 22A

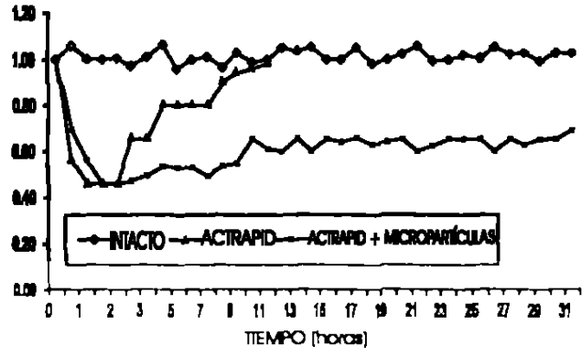

FIG. 22B

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Sheet No. 1

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

. .. receiving Office use only
International Application No.
International Filing Date
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(If desired) (12 characters maximum)* 028093-0110

Box No. I TITLE OF INVENTION *DELIVERY SYSTEM FOR DRUG AND CELL THERAPY*

Box No. II APPLICANT ☐ This person is also inventor.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

THE TECHNOLOGY DEVELOPMENT COMPANY LTD.
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Teleprinter No.

Applicant's registration No. with the Office

State *(that is, country)* of nationality: RU

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This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: RU

State *(that is, country)* of residence: RU

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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☐ *Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.*

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Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

1. If, in any of the Boxes, except Boxes Nos. VIII(i) to (v) for which a special continuation box is provided, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ... " (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;

2. If the applicant intends to make an indication of the wish that the international application be treated, in certain designated States, as an application for a patent of addition, certificate of addition, inventor's certificate of addition or utility certificate of addition: in such a case, write the name or two-letter code of each designated State concerned and the indication "patent of addition, "certificate of addition, "inventor's certificate of addition" or "utility certificate of addition," the number of the parent application or parent patent or other patent grant and the date of grant of the parent patent or other patent grant or the date of filing of the parent application (Rules 4.11(a)(iii) and 49bis.1(a) or (b)).

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Continuation of Box IV

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The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.

However,

☐ **DE Germany is not designated for any kind of national protection**

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(The check-boxes above may be used to exclude (irrevocably) the designations concerned in order to avoid the ceasing of the effect, under the national law, of an earlier national application from which priority is claimed. See the Notes to Box No. V as to the consequences of such national law provisions in these and certain other States.)

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application (*day/month/year*)	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (1) 04 March 2003 (04/03/2003)	60/451,245	US		
item (2) 05 May 2003 (05/05/2003)	60/467,601	US		
item (3) 09 May 2003 (09/05/2003)	60/469,017	US		

☒ **Further priority claims are indicated in the Supplemental Box.**

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (*only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office*) identified above as:

☒ **all items** ☐ **item(1)** ☐ **item(2)** ☐ **item(3)** ☐ **item(4)** ☐ **item(5)** ☐ **other, see Supplemental Box**

* ***Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):*** .....

.....

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) (***if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used***):

ISA/EP

Request to use results of earlier search; reference to that search (*if an earlier search has been carried out by or requested from the International Searching Authority*):

Date (*day/month/year*) Number Country (*or regional Office*)

Box No. VIII DECLARATIONS

The following declarations are contained in Boxes Nos. VIII (i) to (v) (*mark the applicable check-boxes below and indicate in the right column the number of each type of declaration*):		**Number of declarations**
☐ **Box No. VIII(i)**	Declaration as to the identity of the inventor :	
☐ **Box No. VIII(ii)**	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent :	
☐ **Box No. VIII(iii)**	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application :	
☐ **Box No. VIII(iv)**	Declaration of inventorship (only for the purposes of the designation of the United States of America) :	
☐ **Box No. VIII(v)**	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty :	

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SUPPLEMENTAL BOX
Continuation of Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:				
Filing date of earlier application (*day/month/year*)	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (4) 15 August 2003 (15/08/2003)	60/495,097	US		
item (5)				
item (6)				

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Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application contains:

(a) In paper form, the following number of sheets:

request (including declaration sheets)	:	5
description (excluding sequence listings and/or tables related thereto)	:	54
claims:	:	13
abstract	:	1
drawings	:	18
Sub-total number of sheets	:	91
Sequence listings	:	
tables related thereto	:	
(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c) below)		
Total number of sheets	:	91

(b) ☐ only in computer readable form (Section 801(a)(i))
- (i) ☐ sequence listings
- (ii) ☐ tables related thereto

(c) ☐ also in computer readable form (Section 801(a)(ii))
- (i) ☐ sequence listings
- (ii) ☐ tables related thereto

Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the
- ☐ sequence listings:
- ☐ tables related thereto

(additional copies to be indicated under items 9(ii) and/or 10(ii), in right column):

This international application is accompanied by the following item(s) *(mark the applicable check-boxes below and indicate in right column the number of each item)*: — Number of items

1. ☒ fee calculation sheet : 1
2. ☐ original separate power of attorney :
3. ☐ original general power of attorney :
4. ☐ copy of general power of attorney; reference number, if any..... :
7. ☐ statement explaining lack of signature :
6. ☐ priority document(s) identified in Box No. VI as item(s):..... :
7. ☐ translation of international application into (*language*):..... :
8. ☐ separate indications concerning deposited microorganism or other biological material :
9. ☐ sequence listings in computer readable form *(indicate type and number of carriers)*
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11. ☒ other (*specify*): Transmittal Letter, Postcard & Check..... :3

Figure of the drawings which should accompany the abstract: 1

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Michael D. Kaminski

Michael D. Kaminski, Agent for Applicants

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1. Date of actual receipt of the purported international application:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid

2. Drawings:
- ☐ received:
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FEE CALCULATION SHEET

Annex to the Request

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Date stamp of the receiving Office

Applicant's or agent's file reference 028093-0110

Applicant
THE TECHNOLOGY DEVELOPMENT COMPANY LTD., et al

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE ..... 300 T

2. SEARCH FEE ..... 1818 S

International search to be carried out by EP
(If two or more International Searching Authorities are competent to carry out the international search, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FILING FEE

Where items (b) and/or (c) of Box No. IX apply, enter Sub-total number of sheets
Where items (b) and (c) of Box No. IX do not apply, enter Total number of sheets } ____

The international application contains 91 sheets.

i1 first 30 sheets 1035 i1

i2 61 x 11 = 671 i2

i3 additional component (only if sequence listing and/or tables related thereto are filed in computer readable form under Section 801(a)(i), or both in that form and on paper, under Section 801(a)(ii)):

x ____ = $ 0.00 i3

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4. FEE FOR PRIORITY DOCUMENT *(if applicable)* ..... 80 P

5. TOTAL FEES PAYABLE ..... 3904 TOTAL
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M DE OF PAYMENT

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Receiving Office: RO/ US

Deposit Account No: 19-0741

Date: 04 March 2004

Name: Michael D. Kaminski
(Reg. No. 32,904)

Signature: Michael D. Kaminski

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DELIVERY SYSTEM FOR DRUG AND CELL THERAPY

FIELD OF THE INVENTION

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

[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. Patent 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. Patent 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] Figure 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] Figure 2A is a photograph of a cross-section of crystallized dextran microparticles shown in Figure 1.

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

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

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

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

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

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

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

[0016] Figure 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 14th day after intermuscular injection.

[0017] Figure 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] Figure 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 Figure 1.

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

[0020] Figure 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 Figure 1.

[0021] Figure 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 Figure 1.

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

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

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

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

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

[0027] Figures 22A and 22B 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 Figures 2A, 2B). 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 Figure 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 microparticles, 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] Figures 4 and 5 show the implant in tissue following subcutaneous (Figure 4) and intra-muscular (Figure 5) injections in experimental animals (mice). No inflammation reactions were detected in the animal's tissue during 180 days.

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

[0036] Figures 8A, 8B and 8C show the implant on the 4th, 11th and 28th day, respectively, after the subcutaneous injection. Figures 8D and 8E both show the implant 180 days after the subcutaneous injection. Figures 8F and 8G both show the implant one year after the

subcutaneous injection. As shown in Figure 8G, 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] Figures 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 14th day after the intermuscular injection (Figure 9) and expression of the reporter gene in a mouse's muscle tissue following the plasmid DNA release from the implant (Figure 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 surrounded 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, publs. 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 Figures 11 and 12.

[0045] Figure 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 Figure 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] Figure 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 Figure 12, the smaller volume (droplet) dextran phase forms into a large spherical dextran / dextran microparticle core (bottom right of Figure 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 Figures 13 and 14. The volume of the dextran phase is greater than the volume of the PEG phase in the two-phase system. Both Figures 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.

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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. Figure 15A 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 Figure 15A. 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 Figure 15A, 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 Figure 15A, 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] Figure 15B illustrates a scanning electron microscope image of a cross section of an implant structure based on the two phase system schematically illustrated in Figure 15A. 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 Figure 15B 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 Figure 15B forms by self assembly as shown schematically in Figure 15C. 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 Figure 15C. 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 Figure 15C. 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 Figures 3 and 12) or absorbed by the same surface being inside of macro-porous structures (see Figure 11) or capsules (see Figures 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, Figures 16A-16D illustrate a partition of HeLa cells and crystallized dextran microparticles in a dextran-PEG two phase system. In Figure 16A, 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. Figure 16B 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. Figures 16C and 16D 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] Figure 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 Figure 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 Figure 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 Figure 18. Furthermore, as shown in Figure 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 Figure 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

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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 Figure 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. Figure 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, CA, USA) after proper calibration.

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[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 GL, Lee HJ, 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 Figure 19, a two phase composition, described above and illustrated in Figures 13, 14, 15A, 15B, 15C and

16B 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 Figure 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. Figure 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. Figure 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, MA) 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, CA, 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 #		0min glucose mmol/l	15min glucose mmol/l	30min glucose mmol/l	45min glucose mmol/l	120min glucose mmol/l	210min glucose mmol/l	270min glucose mmol/l	390min 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 crystal-lized dextran micro-particles	6.0	4.3	3.2	2.5	0.8	0.8	0.9	0.7
19	Insulin 0.5 UI with crystal-lized dextran micro-particles	6.9	5.6	4.1	3.4	-	1.2	-	1.6
20	Insulin 0.5 UI with crystal-lized dextran micro-particles	5.9	3.5	2.9	1.9	1.2	1.0	1.0	0.7
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 Figures 22A and 22B, 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 (Figure 22B) and about seventeen to about twenty-four hours for "Monotard HM"® insulin alone (Figure 22A). 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 Figures 22A and 22B, the upper line illustrates the control line for intact rabbits to which no insulin was administered. The y-axis of Figures 22A and 22B 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 Figures 22A and 22B 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, CA, 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/PIs/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 (Fluka) 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, semi-synthetic 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

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 solution, 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 microparticles;

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

5. The composition of claims 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 claims 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.

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.

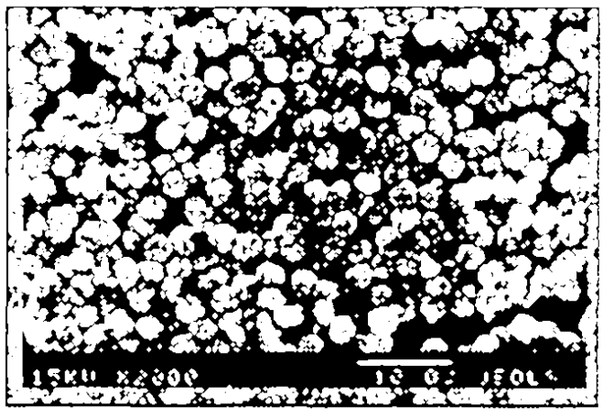

FIG. 1

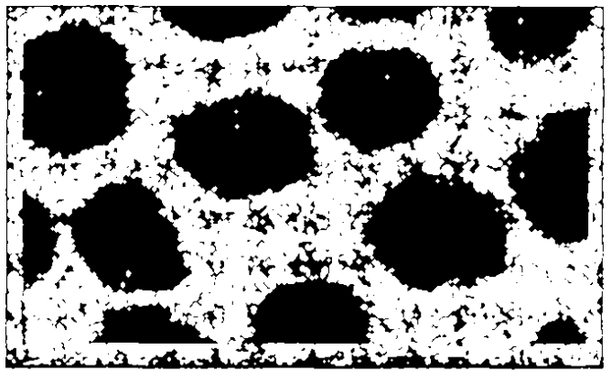

FIG. 2A

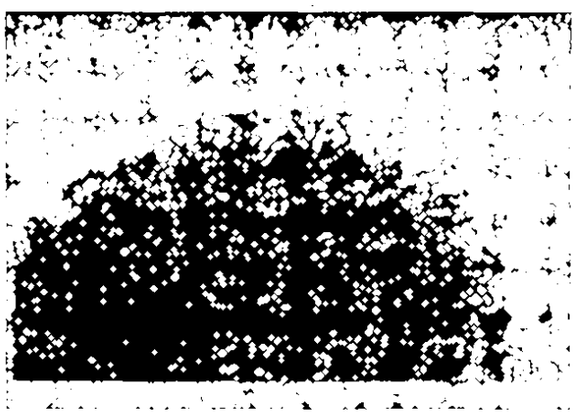

FIG. 2B

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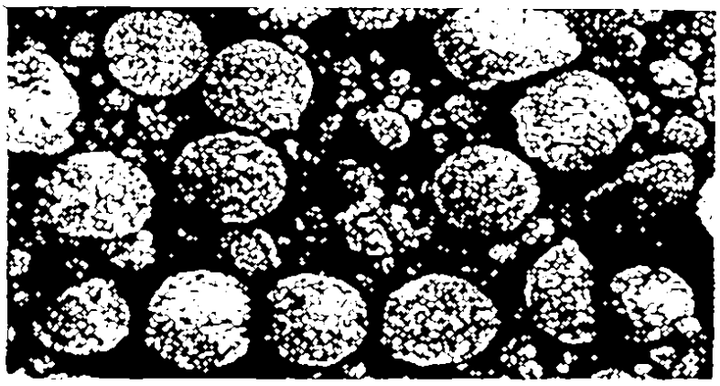

FIG. 3

FIG. 4

FIG. 5

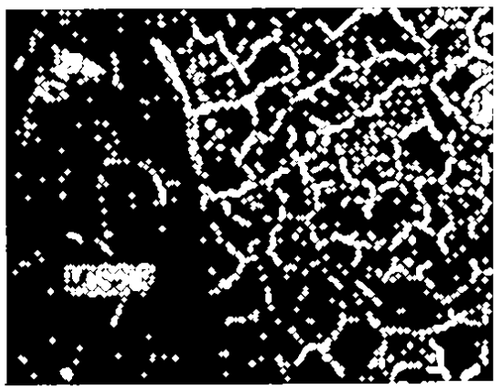

FIG. 6A

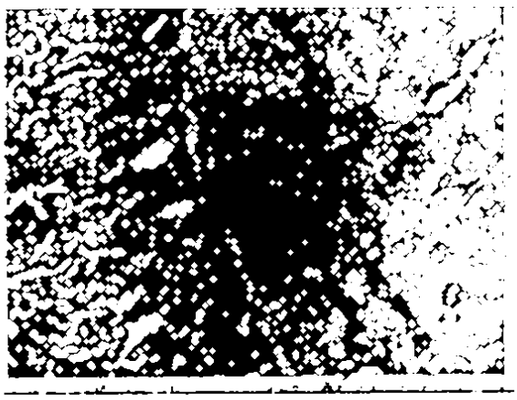

FIG. 6B

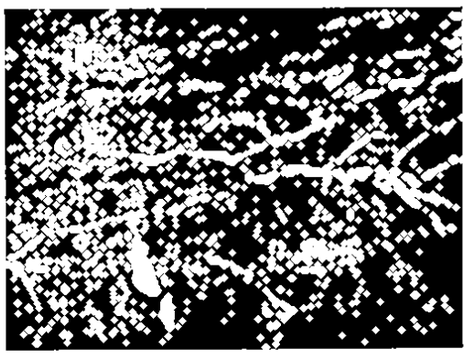

FIG. 6C

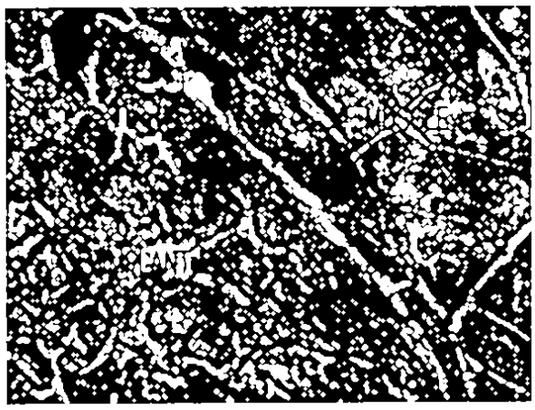

FIG. 7A

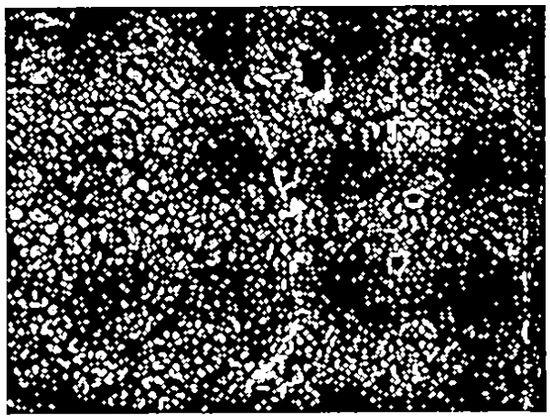

FIG. 7B

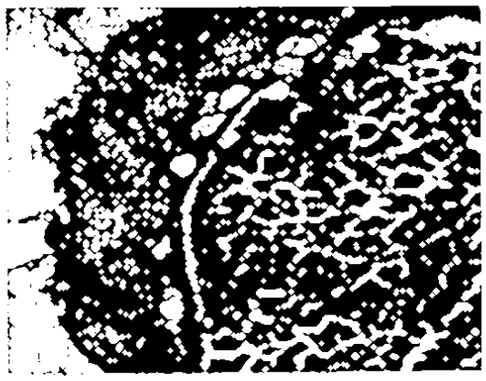

FIG. 8A

FIG. 8B

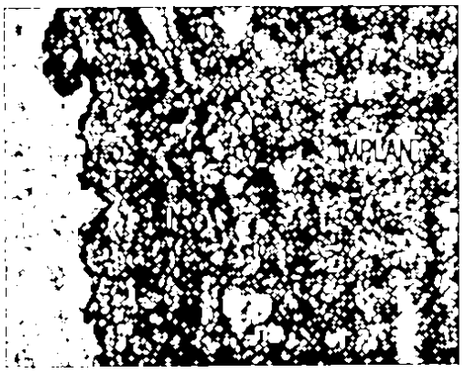

FIG. 8C

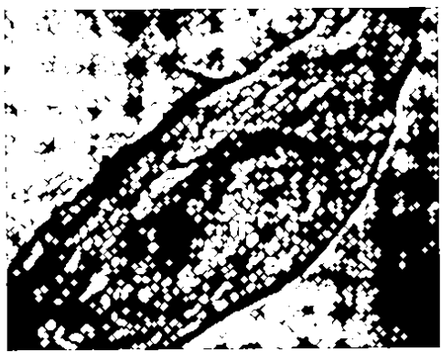

FIG. 8D

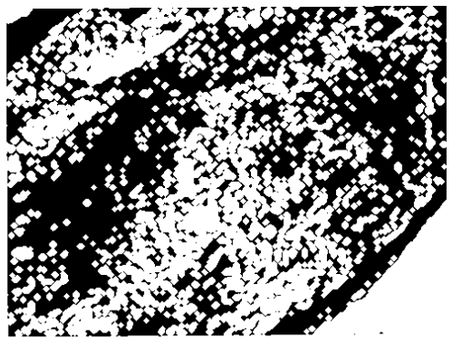

FIG. 8E

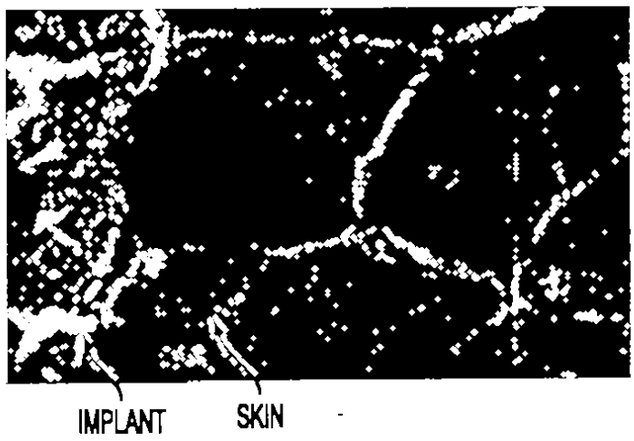

FIG. 8F

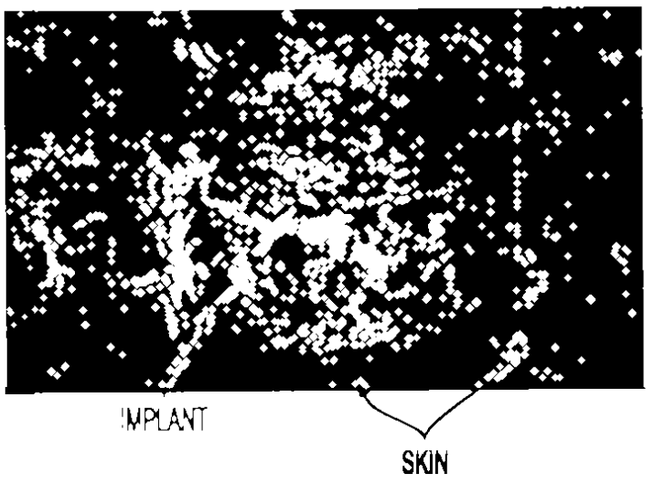

FIG. 8G

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FIG. 9

FIG. 10

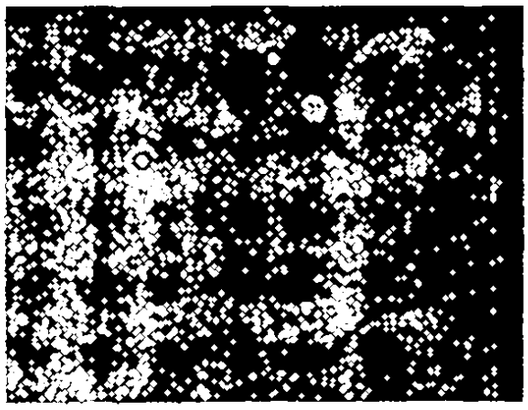

FIG. 11

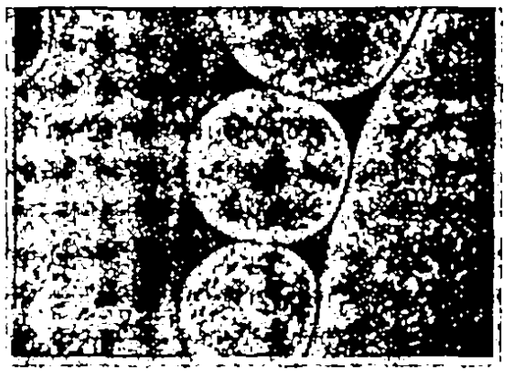

FIG. 12

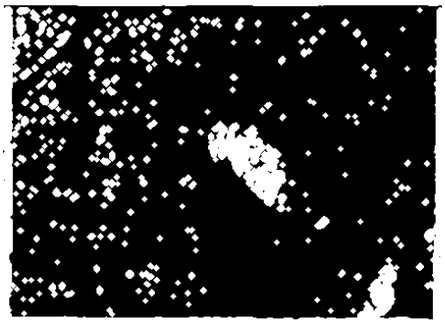

FIG. 13

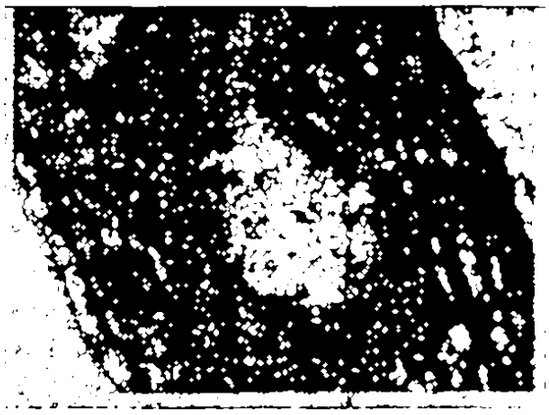

FIG. 14

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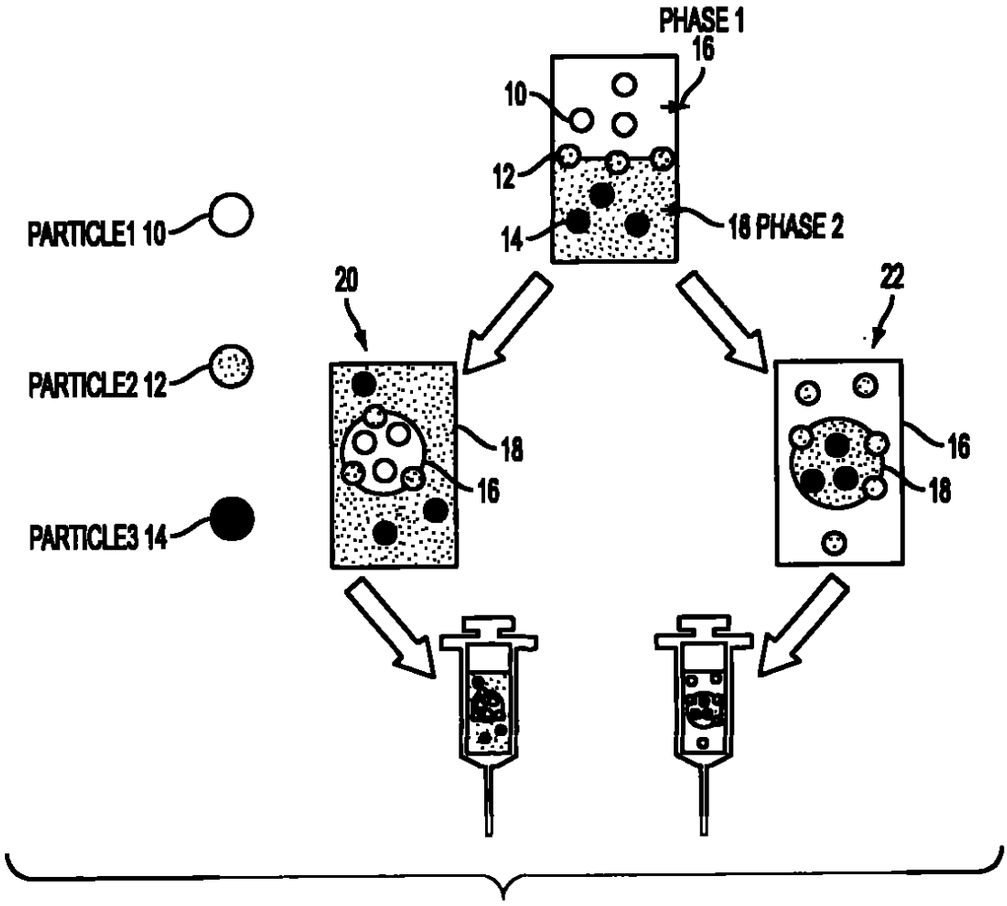

FIG. 15A

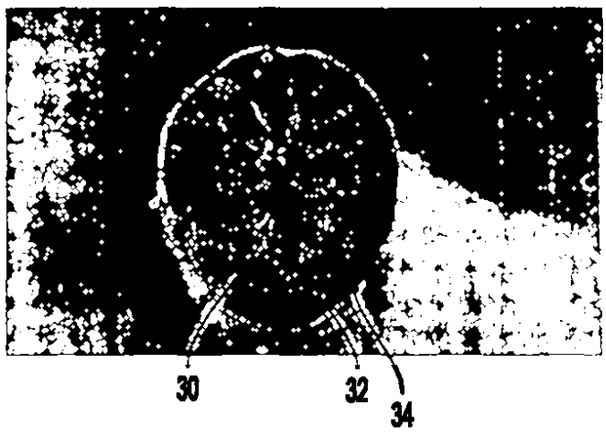

FIG. 15B

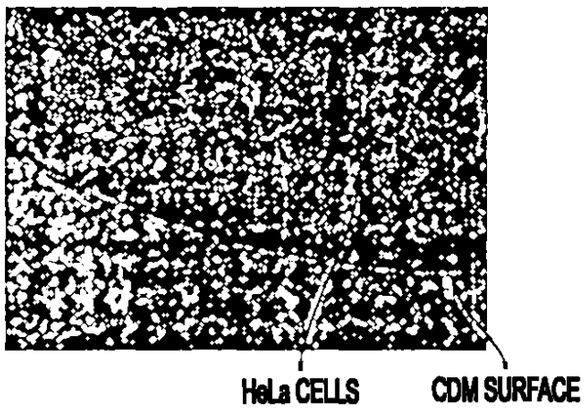

FIG. 16A

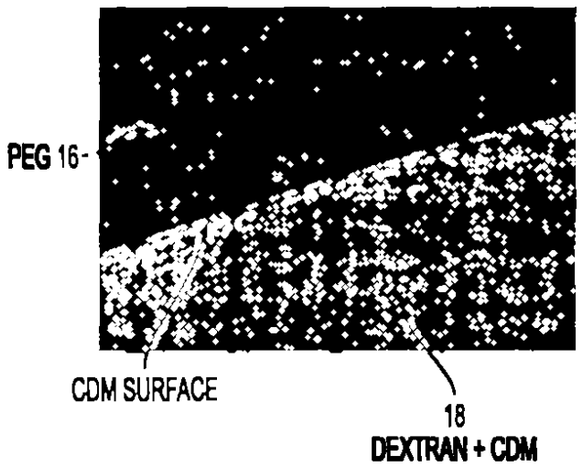

FIG. 16B

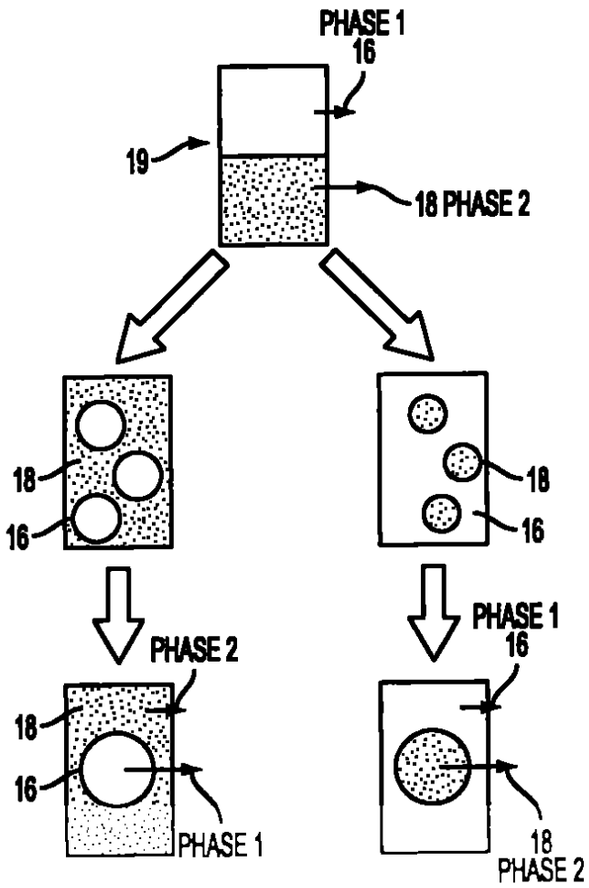
PHASE 1
16
19
18 PHASE 2
18
16
18
16
PHASE 2
18
16
PHASE 1
PHASE 1
16
18
PHASE 2

FIG. 15C

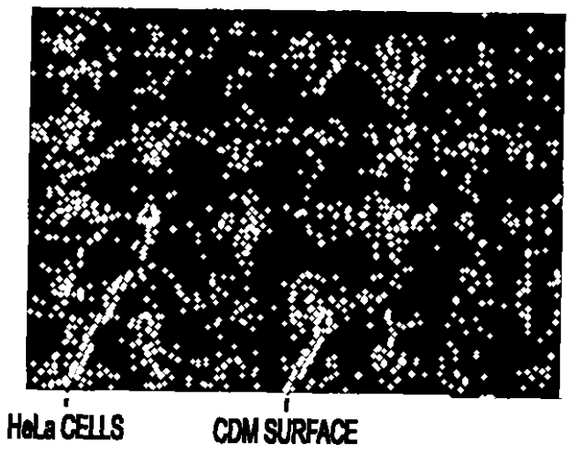

FIG. 16C

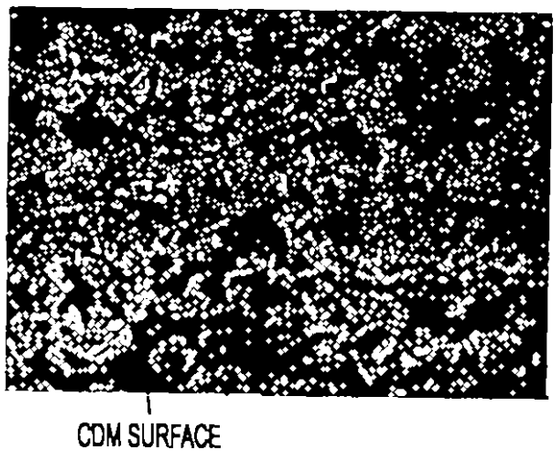

FIG. 16D

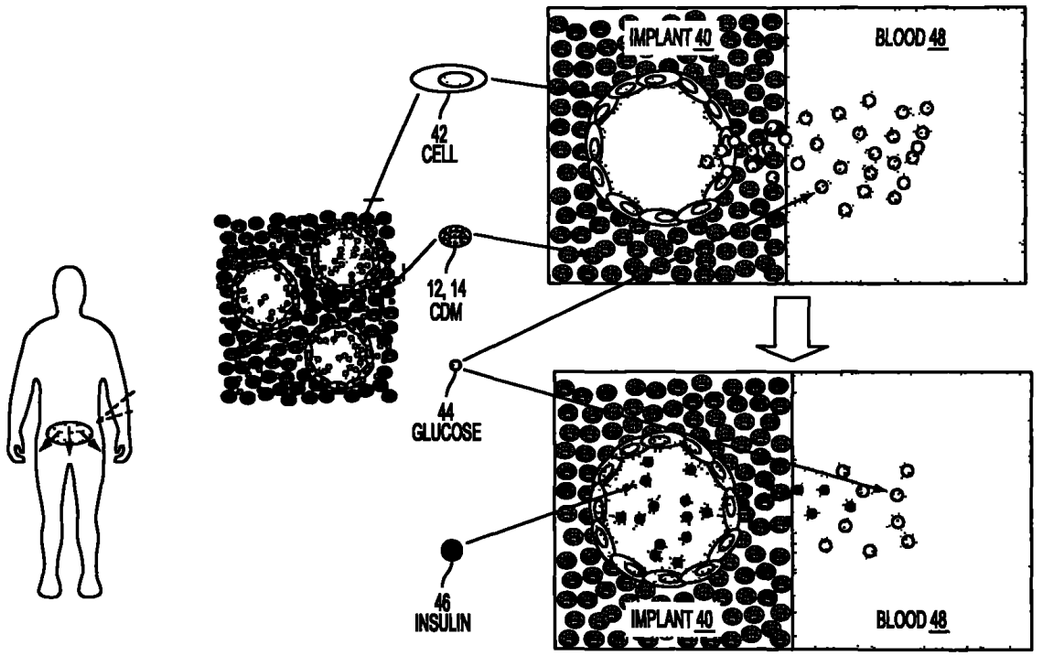
IMPLANT 40
BLOOD 48
42
CELL
12, 14
CDM
44
GLUCOSE
46
INSULIN
IMPLANT 40
BLOOD 48

FIG 17

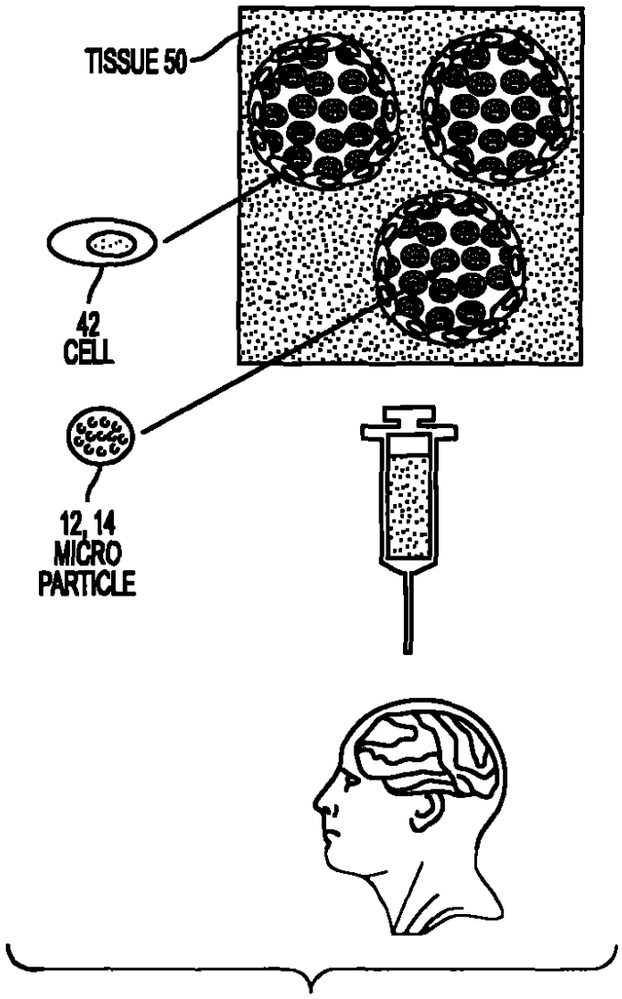

FIG. 18

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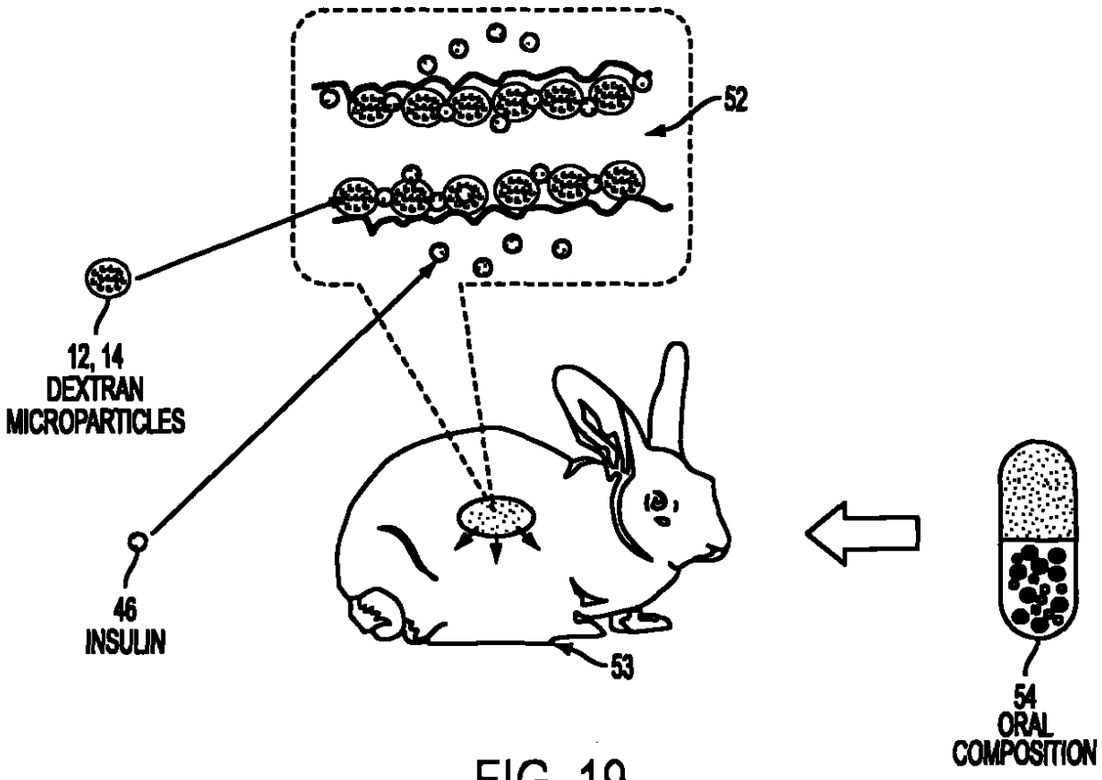
52
12, 14
DEXTRAN
MICROPARTICLES
46
INSULIN
53
54
ORAL
COMPOSITION

FIG. 19

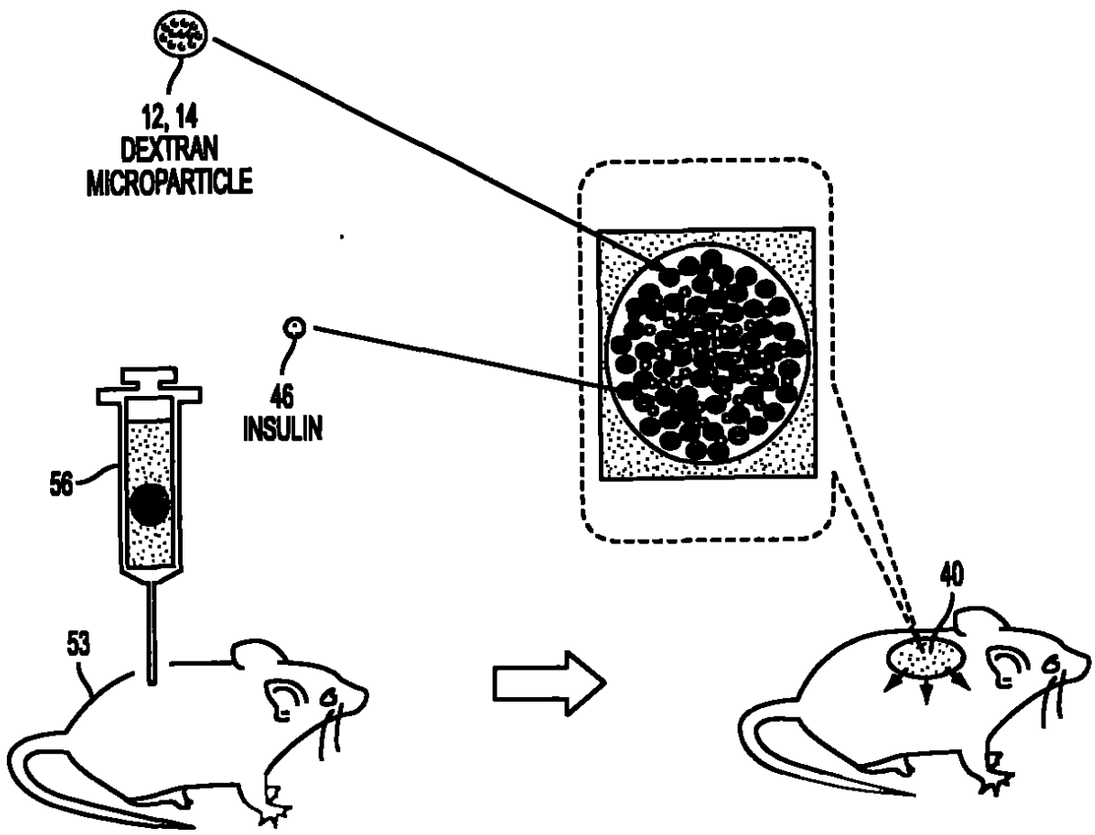

FIG. 20

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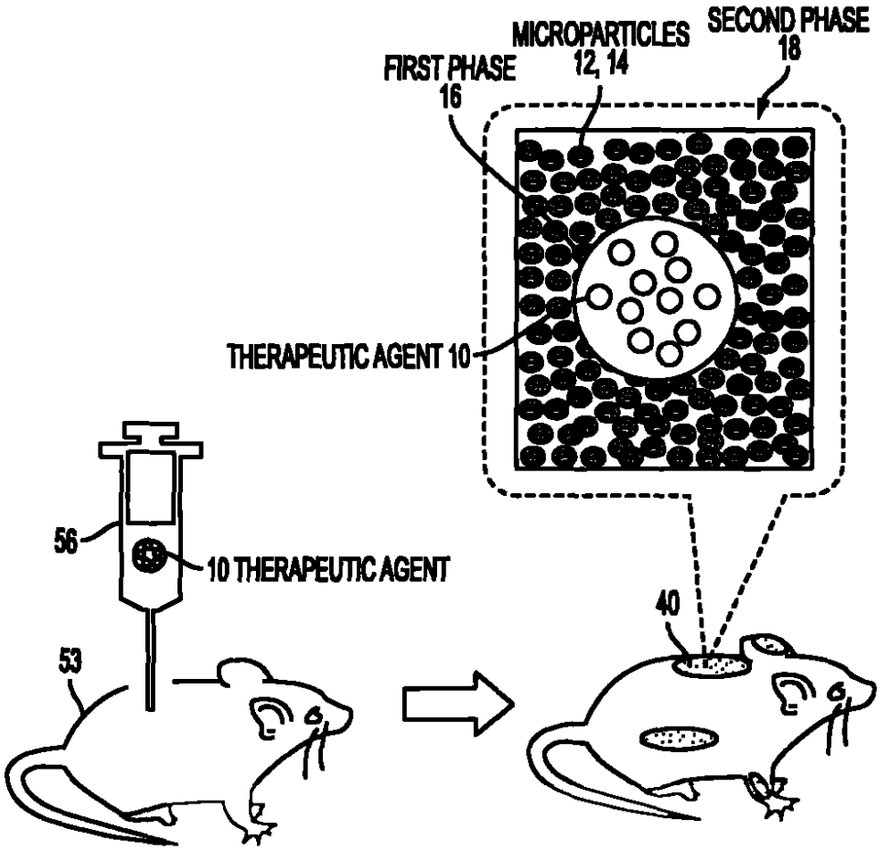
MICROPARTICLES
12, 14
SECOND PHASE
18
FIRST PHASE
16
THERAPEUTIC AGENT 10
56
10 THERAPEUTIC AGENT
53
40

FIG. 21

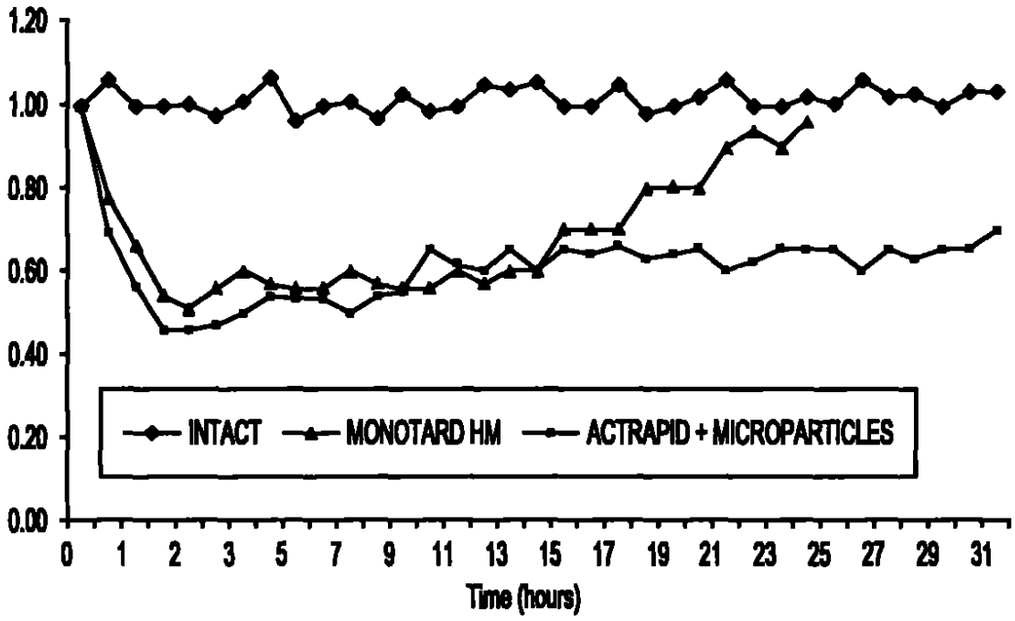

FIG. 22A

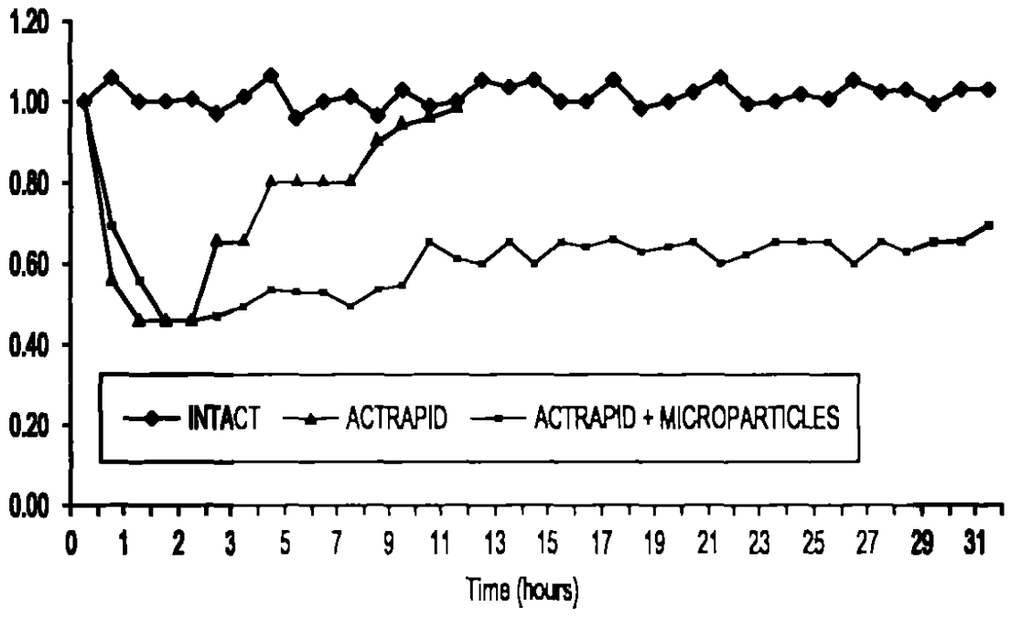

FIG. 22B

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

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PCT

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60/495,097	15 August 2003 (15.08.2003)	US

(71) Applicant *(for all designated States except US)*: THE TECHNOLOGY DEVELOPMENT COMPANY LTD. [RU/—]; Reid House, 31 Church Street, Hamilton HM FX (BM).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: SABETSKY, Vladimir [RU/RU]; pr. Piskarevsky, 10 q.320, Saint-Petersburg, 105221 (RU).

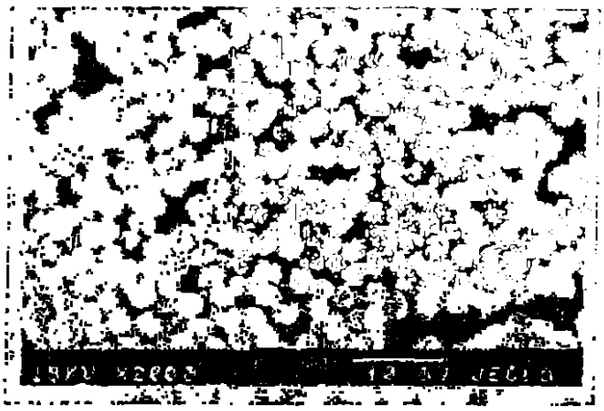
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,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

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

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DELIVERY SYSTEM FOR DRUG AND CELL THERAPY



(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.

WO 2004/078197 A1

Atty. Dkt. No. 028093/0110

IN THE EUROPEAN PATENT OFFICE
INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY
UNDER THE PATENT COOPERATION TREATY

International Application of:	THE TECHNOLOGY DEVELOPMENT COMPANY
International Application No:	PCT/IB2004/000961
International Filing Date:	4 March 2004 (04.03.2004)
Title:	DELIVERY SYSTEM FOR DRUG AND CELL THERAPY

REPLY TO THE WRITTEN OPINION UNDER PCT RULE 66.3 and ARTICLE 34 AMENDMENT

European Patent Office
P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk

Authorized Officer: Sandrine POLENZANI

Sir:

In accordance with PCT Rule 66.3, Applicant provides a Reply to the Written Opinion.

AMENDMENTS

Please amend claim 1 by replacing claim page 55 of the published application with replacement page 55 submitted with this Reply to Written Opinion.

ARGUMENTS

The Written Opinion asserts that all claims either lack novelty under Article 33(2) and/or lack inventive step under Article 33(3). Applicant respectfully disagrees for the following reasons.

I. Novelty

Claim 1

Claim 1 has been amended to recite a composition capable formation of a three dimensional structured object in vivo. In order for claim 1 to be rendered not novel according to PCT article 33(1), the compositions described in D1 – D4 must be at least capable of forming a three dimensional structured object in vivo. Applicant submits that the compositions disclosed in D1 to D4 are not capable of forming such an object in vivo. Therefore, claim 1 satisfies the novelty requirement.

Reference D1 discloses an in vitro realized process of micro-encapsulation. For example, claim 1 of D1 recites "A process for producing parenterally administrable microparticles containing a biologically active substance...". Application of two-phase polymer system shown is only to form in vitro droplets of aqueous starch solution containing washed "...concentrated and/or solidified biologically active substance..." and "causing or allowing the starch droplets ... to solidify into starch microparticles". This reference does not form any structured objects in vivo.

Reference D2 discloses a composition based on specific type of block-copolymers particles (= "suspended carrier particles") "...capable of associating with at least one active principal (AP) in colloidal suspension, in the undissolved state, and releasing it, especially in vivo, in a prolonged and/or delayed manner, characterized in that the particles it contains are associated and/or can be associated with at least one AP selected from hydrophilic AP, preferably proteins, this AP consisting particularly preferably of insulin." Thus, this reference does not disclose forming a structured object in vivo.

Reference D3 notes that "... important feature of this invention is the protection afforded the insulin component of this composition from enzymatic degradation in the digestive tract. This is accomplished by incorporating the insulin in the coacervate phase of

the coacervate system. In the process of this incorporation, a film of coacervate phase water surrounds and coats each insulin molecule." The emulsifying procedure forms an emulsion containing particles in vitro (see example 1 in col. 7 of D3).

Therefore, Reference D3 does not disclose a composition which can form a 3D structure in vivo as recited in claim 1.

Reference D4 describes a technique that uses special derivatives of dextran that can form two-phase system with PEG in aqueous solution and can be cross-linked to obtain a dextran hydrogel with PEG-phase containing insulin and dispersed in the hydrogel (i.e. the droplets are fixed and cannot move in the gel). The dextran hydrogel is not flowable system.

In contrast, claim 1 of the present application recites a flowable composition. A preferred example of the composition recited in claim 1, does not include dextran derivatives and cross-linking agents. Droplets of the PEG-phase containing insulin are dispersed in a dextran phase containing crystallized dextran microparticles. The whole system is flowable and evolves to form a specific 3D structure in vivo.

Therefore, Claim 1 is believed to be novel because references D1-D4 do not disclose or suggest any flowable colloidal systems capable of forming a specifically structured object in vivo.

Claim 2

The Written Opinion states that it " is stressed that the feature "when the composition is provided into a mammal body" relates merely to the process of preparation and is not a characterizing feature in a claim directed to a product"

Applicant respectfully disagrees with this conclusion. In order for claim 2 to be rendered not novel according to PCT article 33(1), the compositions described in D3 and D4

3HE
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must be at least capable of self assembling a wall when provided into a mammal body (i.e., in vivo). Applicant submits that the compositions disclosed in D3 and D4 are not capable of forming such a wall in vivo. Therefore, claim 2 satisfies the novelty requirement.

Claim 2 recites **self assembling** as a basis of in vivo specific structure formation - **"...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."**

The in vivo structure formation process of claim 2 is of a type where a solution of the biodegradable polymer in an organic solvent and a therapeutic agent dissolved or dispersed (in this case composition is a suspension or emulsion, i.e. it is a colloidal system) in the same solvent is provided into a body. Upon contact with body fluid (i.e. when the composition is provided into a mammal body), the polymer coagulates, forming a solid implant that can be used as depot for controlled release of biologically active substance(s).

References D3 and D4 do not disclose such a composition or a composition which is capable of forming the claimed structure.

Claim 27

Applicant submits that reference D3 lacks page 238 mentioned in the Written Opinion. D3 does not disclose FITC labeled insulin. Thus, Applicant believes that claim 27 is novel over D3.

Applicant notes that reference D4 (Moriyama article) does have page 238 and does disclose FITC labeled insulin. However, D4 discloses a cross linked hydrogel, which is not a flowable composition. In contrast, claim 27 recites a flowable composition. Thus, claim 27 is novel over D4 as well for this reason.

Claim 43

Reference D3 (US patent 4,963,526) does not disclose crystallized dextran microparticles and combining the microparticles with insulin after the microparticles are formed, as recited in claim 43. Instead, gelatin, lecithicin, albumin, etc. is used with insulin. Likewise, D4 discloses cross linked rather than the crystallized microparticles of claim 43.

Claim 51

Claim 51 recites crystallized dextran microparticles. In contrast, D4 discloses cross linked dextran rather than crystallized microparticles. Furthermore, the cross linked hydrogel of D4 is formed after dextran is mixed with insulin (i.e., insulin is mixed with microparticles before cross linking of the dextran) (see § 2.2 and § 2.3 of D4). In contrast, claim 51 recites that insulin is mixed with microparticles after the microparticles form.

Claim 54

The Written Opinion States that the feature "the microparticles are formed prior to combination of the insulin and the microparticles" does not delimit claim 54 from the prior art. A product is not delimited from the prior art only when alternative process is used to prepare a known product. Consequently, claim 54 is not new in view of documents D4 and D5."

Applicant respectfully disagrees with this conclusion. In order for claim 54 to be rendered not novel according to PCT article 33(1), the compositions described in D4 and D5 must be the same as the composition of claim 54.

Reference D4 describes the insulin release during cross-linked methacrylic dextran hydrogel enzymatic degradation. In contrast, claim 54 recites crystallized dextran. Clearly, the cross linked dextran hydrogel is not the same as the claimed crystallized dextran.

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Furthermore, D4 discloses release based on the enzymatic degradation. Clearly, this excludes introducing insulin into a cross-linked hydrogel following the preparation of the hydrogel. Thus, the composition of D4 is different from the claimed composition.

Reference D5 does disclose crystallized dextran particles. However, in the composition of D5, the insulin is introduced into the composition prior to formation of the microparticles. Thus, the composition of D5 includes insulin surrounded by a dextran particle shell, since the shell forms around the insulin.

In contrast, in the composition of claim 54, the insulin is located in the pores of crystallized dextran microparticles because the insulin is provided into the microparticles after the microparticles are formed. Claim 56 specifically recites that the insulin is located in the pores of the microparticles but is not encapsulated in the dextran shell to further distinguish D5.

Therefore, the composition of claims 54 and 56 is structurally different from the composition of D5 where the insulin is surrounded by a dextran shell.

Claims 58-61

As discussed above, D4 does not disclose the composition of claim 58. Furthermore D4 does not disclose the compositions of claims 59-61 because the composition of D4 is not a flowable composition, as recited in claim 59.

II. Inventive Step

Claims 14-15 and 30

The Written Opinion states that "document D4 discloses a process for preparing dextran-PEG two phase compositions comprising insulin eventually labeled with FITC. Claims 14-15 and 30 differ from D4 in that the colloidal system or two phase composition is formed in vivo. The problem to be solved is thus regarded as to provided a process for preparing the composition in vivo. There is no evidence provided in the application that the colloidal system of two phase composition may be formed in vivo. The problem is thus not solved and claims 14-15 and 30 are not inventive."

Applicant respectfully disagrees with this conclusion. The solution to the problem is shown in Figures 13 and 14 and described in paragraph 0049 of the present application. Figures 13 and 14 show how the colloidal system of a two phase composition forms an in vivo implant of claims 14-15 and 30 following intramuscular and subcutaneous administration of the composition in mice by injections. Thus, the problem is solved and claims 14-15 and 30 are considered inventive.

Claims 33, 38 and 40

The Written Opinion states that there is no evidence provided showing that crystallized dextran microparticles are suitable for cell therapy or as bone graft substitute. Claims 33, 38, and 40 are thus considered as not inventive.

Applicant respectfully disagrees with this conclusion. Partitioning into the PEG-phase (see Figure 16A), adsorption and proliferation of the cells on the surface of the crystallized dextran microparticles partitioned into dextran-phase (see Figures 16C, 16D) and the formation of the two phase composition in vivo (Figures 13-14) taken together show that the technique described in present application can be used for injectable tissue engineering,

which is one of the most promising directions in cell therapy. Thus, claims 33, 38 and 40 are considered inventive.

Claim 47

The Written Opinion states that document D7 discloses that the crystallization is spontaneous above 4°C and a higher temperature. The crystallization at temperature above room temperature is an obvious alternative to process disclosed in D7. Claim 47 does thus not involve an inventive step.

Applicant respectfully disagrees with this conclusion. Document D7 states: "Surprisingly, it was furthermore found that the crystallization rate lowered at 4°C. This finding can be used to store solutions at 4°C while these solutions only start to form gels when they are removed from the cooled storage and are brought to higher temperature such as room temperature."

Crystallization at temperature above one disclosed in D7 (i.e. 25°C) is not obvious because it is well known scientific fact that the increasing of temperature impedes the crystallization process. It is why the authors of D7 say "Surprisingly ..." when crystallization rate was lowered at 4°C because it is well known scientific fact that lowering of temperature favours crystallization process. There is no suggestion in D7 that the crystallization can be conducted above room temperature. Thus, claim 47 is considered inventive because the present application shows that crystallization is possible above room temperature.

Claim 62

Claim 62 is believed to be inventive for at least the same reason as claim 54 above. References D4 to D6 do not disclose a composition is which the insulin is provided after the formation of the microparticles, such that the composition would comprise insulin in pores of the microparticles, for example.

Claim 67

The Written Opinion states that the application does not provide any evidence that the crystallized dextran microparticles are suitable for inhalation administration. An inventive step is thus not acknowledged for the subject-matter of claim 67.

Applicant respectfully disagrees with this conclusion. Crystallized dextran microparticles dimensions (see Figure 1 and paragraph 0028 of the specification), their biocompatibility and possibility of acting as a carrier of biologically active substances undoubtedly show that the microparticles have a sufficient set of properties to be applied by inhalation and claim 67, therefore, is inventive.

Claim 68

Claim 68 which recites administration by inhalation is believed to be inventive for the same reason as claim 67 above.

The IPEA is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Michael Onischenko
Vice-President
The Technology Development Company, LTD

Dated: 28 December 2004

The Technology Development Company, LTD
c/o FOLEY & LARDNER LLP
Washington Harbour
3000 K Street, N.W., Suite 500
Washington, D.C. 20007-5143
United States of America

AMENDED SHEET 55 FOLLOWS

I claim:

1. A pharmaceutical flowable composition comprising biodegradable and biocompatible components for being provided within or upon a mammal body and adapted for in vivo 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 microparticles;

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

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PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

To:
FOLEY & LARDNER LLP
Attn. Kaminski, Michael D.
Washington Harbour
3000 "K" Street, N.W., Suite 500
Washington, D.C. 20007-5101
UNITED STATES OF AMERICA

Date of mailing (day/month/year)	27/07/2004
Applicant's or agent's file reference: 028093-0110	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No.: PCT/IB2004/000961	International filing date (day/month/year) 04/03/2004
Applicant: THE TECHNOLOGY DEVELOPMENT COMPANY LTD.	

1. [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

Shortly after the expiration of 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months.

See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Sandrine Polenzani

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
028093-0110		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IB2004/000961	04/03/2004	04/03/2003
Applicant		
THE TECHNOLOGY DEVELOPMENT COMPANY LTD.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 05 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      [ ] The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. [X] Certain claims were found unsearchable (See Box II).

3. [ ] Unity of invention is lacking (see Box III).

4. With regard to the title,
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the abstract,
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regards to the drawings,
   a. the figure of the drawings to be published with the abstract is Figure No. 1
      [X] as suggested by the applicant.
      [ ] as selected by this Authority, because the applicant failed to suggest a figure.
      [ ] as selected by this Authority, because this figure better characterizes the invention.
   b. [ ] none of the figures is to be published with the abstract.

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INTER. ONAL SEARCH REPORT

International Application No
PCT/IB2004/000961

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/28 A61K47/36 A61K9/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/39985 A (LAAKSO TIMO ; RESLOW MATS (SE); BIOGLAN AB (SE); JOENSSON MONICA (SE)) 23 May 2002 (2002-05-23) page 19, line 21 - page 20, line 10 page 19, line 29 - line 36	1-11,13
X	WO 02/28374 A (BRYSON NATHAN ; FLAMEL TECH SA (FR)) 11 April 2002 (2002-04-11) page 1, line 16 - line 21 page 9, line 14 - line 27	1
X	US 4 963 526 A (ECANOW BERNARD) 16 October 1990 (1990-10-16) claims 1,25	1,2
	-/--	

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

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 July 2004	27/07/2004

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

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MORIYAMA K ET AL: "Regulated insulin release from biodegradable dextran hydrogels containing poly(ethylene glycol)" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 42, no. 3, 1 December 1996 (1996-12-01), pages 237-248, XP004069859 ISSN: 0168-3659 page 240	1-13, 15-29, 43-61
Y	page 238, column 1 - column 2	62-68
X	US 4 713 249 A (SCHROEDER ULF) 15 December 1987 (1987-12-15) cited in the application	47-50, 54-57
Y	column 6; claims 1,4,7; example 13	62-68
X	STENEKES R J H ET AL: "Formation of dextran hydrogels by crystallization" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 22, no. 13, July 2001 (2001-07), pages 1891-1898, XP004245950 ISSN: 0142-9612 page 1892, column 1	47-61
X	EP 1 184 032 A (OCTOPLUS B V) 6 March 2002 (2002-03-06) page 3, paragraphs 15,16 page 4, line 46 - page 5, line 28	47-53
A	WO 99/02107 A (USBIOMATERIALS CORP ; UNIV FLORIDA (US)) 21 January 1999 (1999-01-21) the whole document	38-42
A	WO 87/02704 A (ROBINSON ERIC) 7 May 1987 (1987-05-07) the whole document	33-37
A	SOON-SHIONG P: "Encapsulated islet cell therapy for the treatment of diabetes: Intraperitoneal injection of islets" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 39, no. 2, 1 May 1996 (1996-05-01), pages 399-409, XP004037345 ISSN: 0168-3659 the whole document	33-37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2004/000961

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

Although claims 14-26, 30-37, 40-46, 62-66 and 68 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the composition.

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

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

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

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

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

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

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

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

INTER. ONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB2004/000961

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0239985	A	23-05-2002	SE	518008 C2	13-08-2002
			AU	9252701 A	27-05-2002
			CA	2429100 A1	23-05-2002
			EP	1333814 A1	13-08-2003
			JP	2004513914 T	13-05-2004
			SE	0004218 A	17-05-2002
			WO	0239985 A1	23-05-2002
			US	2002081336 A1	27-06-2002
WO 0228374	A	11-04-2002	FR	2814951 A1	12-04-2002
			AU	9393901 A	15-04-2002
			BR	0114513 A	21-10-2003
			CA	2424981 A1	11-04-2002
			CN	1468095 T	14-01-2004
			EP	1322296 A1	02-07-2003
			WO	0228374 A1	11-04-2002
			JP	2004510729 T	08-04-2004
			US	2004048782 A1	11-03-2004
US 4963526	A	16-10-1990	US	4849405 A	18-07-1989
			EP	0179904 A1	07-05-1986
			IL	75263 A	05-11-1990
			US	4914084 A	03-04-1990
			WO	8505029 A1	21-11-1985
			US	4963367 A	16-10-1990
US 4713249	A	15-12-1987	AU	9127482 A	01-06-1983
			EP	0093757 A1	16-11-1983
			WO	8301738 A1	26-05-1983
			US	4501726 A	26-02-1985
			AT	34659 T	15-06-1988
			AU	567434 B2	19-11-1987
			AU	1776083 A	08-02-1984
			DE	3376797 D1	07-07-1988
			DK	95284 A	23-02-1984
			EP	0113749 A1	25-07-1984
			FI	840923 A	07-03-1984
			NO	840676 A	22-02-1984
			SE	8204244 A	10-01-1984
			WO	8400294 A1	02-02-1984
EP 1184032	A	06-03-2002	EP	1184032 A1	06-03-2002
			AU	9437601 A	13-03-2002
			WO	0217884 A1	07-03-2002
WO 9902107	A	21-01-1999	AU	736846 B2	02-08-2001
			AU	8387498 A	08-02-1999
			BR	9810693 A	15-08-2000
			CA	2295984 A1	21-01-1999
			EP	1009333 A1	21-06-2000
			JP	2001509419 T	24-07-2001
			WO	9902107 A1	21-01-1999
			US	6051247 A	18-04-2000
WO 8702704	A	07-05-1987	EP	0247077 A1	02-12-1987
			WO	8702704 A1	07-05-1987

PATENT COOPERATION TREATY

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From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2004/000961	04.03.2004	04.03.2003

International Patent Classification (IPC) or both national classification and IPC
A61K38/28, A61K47/36, A61K9/00

Applicant
THE TECHNOLOGY DEVELOPMENT COMPANY LTD.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. ***FURTHER ACTION***

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Bochelen, D Telephone No. +49 89 2399-8150

Form PCT/ISA/237 (Cover Sheet) (January 2004)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2004/000961

Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2004/000961

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2004/000961

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 14-26,30-37,40-46,62-66,68

because:

☒ the said international application, or the said claims Nos. 14-26,30-37,40-46,62-66,68 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. -

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ *does not comply with the standard*

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2004/000961

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	14,15,30,33,38,40,47,62,68
	No: Claims	1-2,27,43,51,54,58,67
Inventive step (IS)	Yes: Claims	
	No: Claims	1-67
Industrial applicability (IA)	Yes: Claims	1-13,27-29,38-39,47-61,63-67
	No: Claims	

. Citations and explanations

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/IB2004/000961

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 14-26, 30-37, 40-46, 62-66 and 68 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2. Reference is made to the following documents:

D1: WO 02/39985 A (LAAKSO TIMO ; RESLOW MATS (SE); BIOGLAN AB (SE); JOENSSON MONICA (SE)) 23 May 2002 (2002-05-23)

D2: WO 02/28374 A (BRYSON NATHAN ; FLAMEL TECH SA (FR)) 11 April 2002 (2002-04-11)

D3: US-A-4 963 526 (ECANOW BERNARD) 16 October 1990 (1990-10-16)

D4: MORIYAMA K ET AL: "Regulated insulin release from biodegradable dextran hydrogels containing poly(ethylene glycol)" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 42, no. 3, 1 December 1996 (1996-12-01), pages 237-248, XP004069859 ISSN: 0168-3659

D5: US-A-4 713 249 (SCHROEDER ULF) 15 December 1987 (1987-12-15)

D6: STENEKES R J H ET AL: "Formation of dextran hydrogels by crystallization" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 22, no. 13, July 2001 (2001-07), pages 1891-1898, XP004245950 ISSN: 0142-9612

D7: EP-A-1 184 032 (OCTOPLUS B V) 6 March 2002 (2002-03-06)

D8: WO 99/02107 A (USBIOMATERIALS CORP ; UNIV FLORIDA (US)) 21 January 1999 (1999-01-21)

D9: WO 87/02704 A (ROBINSON ERIC) 7 May 1987 (1987-05-07)

D10: SOON-SHIONG P: "Encapsulated islet cell therapy for the treatment of diabetes: Intraperitoneal injection of islets" JOURNAL OF CONTROLLED

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RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 39, no. 2, 1 May 1996 (1996-05-01), pages 399-409, XP004037345 ISSN: 0168-3659

If not indicated otherwise the relevant passages are those mentioned in the search report.

Document D1 discloses a process for preparing microparticles having incorporated a bioactive substance comprising the step of forming a two-phase system with starch droplets in an inner phase and a polymeric outer phase, e.g. PEG.

Document D2 discloses a colloidal suspension of polymeric microparticles loaded with insulin.

Document D3 discloses a composition for oral administration of insulin comprising a two phase coacervate.

Document D4 discloses heterogenous dextran hydrogels containing PEG and insulin for controlled insulin release.

Document D5 discloses microspheres of crystallized dextran and insulin for prolonged release of biologically active compounds.

Document D6 discloses a process for the preparation of crystallized dextran microspheres by physical cross-linking of aqueous dextran solutions.

Document D7 discloses a process for preparing dextran hydrogels for controlled release systems by physical crystallization of dextran.

Document D8 discloses a composition for replacing tissue like bone, comprising particle of e.g. apatite in a polysaccharidic carrier, e.g. dextran.

Document D9 discloses polymeric microparticles, e.g dextran, for immobilising cells.

Document D9 discloses the use of alginate for encapsulating islet of Langerhans cells for treating diabetes.

3. Novelty (Art. 33(1)(2) PCT):

3.1 Claim 1 relates to a colloidal suspension or emulsion comprising a therapeutic agent. The subject-matter of claim 1 seems to be anticipated by the disclosure of documents D1-D4.

3.2 Claim 2 as far as can be interpreted relates to a two phase composition. Documents D3 and D4 disclose compositions where a phase separation occurs (see D3: claim 25; D4: p238 col1 §2). It is stressed that the feature "when the composition is provided into a mammal body" relates merely to the process of preparation and is not a characterizing feature in a claim directed to a product. It seems thus that claim 2 is not new in view of D3 and D4.

3.3 The prior art does not disclose a process where the colloidal system or the dual phase system eventually with a label is formed in vivo. Claims 14-15 and 30 seem thus to be novel.

3.4 Document D3 discloses FITC labelled insulin in PEG dextran two phase system (see p238 col2). Therefore, claim 27 lacks novelty.

3.5 The prior art does not disclose the use of crystallized dextran microparticles for enclosing or contacting cells for cell therapy. Claim 33 is novel.

3.6 The prior art does not disclose the use of crystallized dextran microparticles for making a bone graft. Claims 38 and 40 are thus novel.

3.7 The method disclosed in D3 anticipates the subject-matter of claim 43.

3.8 The process for preparing crystallized dextran microparticles disclosed in D4 and D6 and D7 is performed at room temperature. The subject-matter of claim 47 is thus novel.

3.9 Claim 51 is not new in view of D4 (see p238 § 2.2).

3.10 The feature "the microparticles are formed prior to combination of the insulin and the microparticles" does not delimit claim 54 from the prior art. A product is not delimited from the prior art only when an alternative process is used to prepare a known product. Consequently, claim 54 is not new in view of documents D4 and D5.

3.11 Claim 58 is not new in view of D4.

3.12 The prior art does not disclose the use of crystallized dextran microparticles for lowering blood glucose in a mammal. Claims 62 and 68 are new.

3.13 The prior art does not disclose an inhalator comprising crystallized dextran microparticles and insulin. Claim 67 is thus novel.

4. Inventive step (Art. 33(1) (3) PCT):

4.1 Document D4 discloses a process for preparing dextran-PEG two phase compositions comprising insulin eventually labelled with FITC. Claims 14-15 and 30 differ from D4 in that the colloidal system or two phase composition is formed in vivo. The problem to be solved is thus regarded as to provide a process for preparing the composition in vivo. There is no evidence provided in the application that the colloidal system or two phase composition may be formed in vivo. The problem is thus not solved an claims 14-15 and 30 are not inventive.

4.2 There is no evidence provided showing that crystallized dextran microparticles are suitable for cell therapy or as bone graft substitute. Claims 33, 38 and 40 are thus considered as not inventive.

4.3 Document D7 discloses that the crystallization is spontaneous above 4°C and a higher temperature. The crystallization at temperature above room temperature is an obvious alternative to process disclosed in D7. Claim 47 does thus not involve an inventive step.

4.4 The use of crystallized dextran microparticles comprising insulin for lowering blood glucose is obvious in view of documents D4-D6. Claims 62 and 68 do thus not involve an inventive step.

4.5 The application does not provide any evidence that the crystallized dextran microparticles are suitable for inhalation administration. An inventive step is thus not acknowledged for the subject-matter of claim 67.

5. Dependent claims 3-13, 16-26,28-29, 31-32, 34-37, 39, 41-42, 44-46, 48-50, 52-53, 55-57, 59-61 and 63-66 do not contain any features which, in combination with the features of any claim to which it/they refers/refer, meet the requirements of the PCT in respect of novelty and/or inventive step, the reasons being as follows:

6. Industrial applicability (Art. 33(1)(4) PCT):

For the assessment of the present claims 14-26, 30-37, 40-46, 62-66 and 68 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII
Certain defects in the international application

7. Although claims 1 and 2; 14-15 and 30; 43, 54 and 58; 47 and 51; 62 and 68 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought. The aforementioned claims therefore lack conciseness and as such do not meet the requirements of Article 6 PCT.

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ASSIGNMENT AND AGREEMENT

WHEREAS, Vladimir SABETSKY of Piskarevsky Prospekt 10, q.320, 105221, Saint-Petersburg, Russia; (hereinafter referred to singly and collectively as "ASSIGNOR") have invented a certain invention entitled **BIOCOMPATIBLE IMPLANT AND METHOD FOR MANUFACTURE THEREOF** (Atty. Dkt. No. 028093-0102) for which a provisional application for United States Letters Patent was filed on March 4, 2003 as Application No. 60/451,245; and

WHEREAS, THE TECHNOLOGY DEVELOPMENT COMPANY Ltd., a Bermuda corporation (hereinafter referred to as "ASSIGNEE") is desirous of acquiring the entire interest therein;

NOW THEREFORE, in consideration of One Dollar ($1.00) and other good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged, ASSIGNOR has sold, assigned, and transferred, and by these presents hereby sells, assigns, and transfers, unto ASSIGNEE, its successors and assigns, the full and exclusive right, title, and interest in and to (a) the above-identified invention or inventions and all improvements and modifications thereof, (b) the above-identified application and all other applications for Letters Patent of the United States and countries foreign thereto for the above-identified invention or inventions and all improvements and modifications thereof, (c) all Letters Patent which may issue from said applications in the United States and countries foreign thereto, (d) all divisions, continuations, reissues, and extensions of said applications and Letters Patent, and (e) the right to claim for any of said applications the full benefits and priority rights under the International Convention and any other international agreement to which the United States adheres; such right, title, and interest to be held and enjoyed by ASSIGNEE, its successors and assigns, to the full end of the term or terms for which any and all such Letters Patent may be granted as fully and entirely as would have been held and enjoyed by ASSIGNOR had this Assignment not been made.

ASSIGNOR HEREBY AUTHORIZES AND REQUESTS the Commissioner of Patents and Trademarks to issue said Letters Patent to ASSIGNEE as assignee of the entire interest, for the sole use and benefit of ASSIGNEE, its successors and assigns.

ASSIGNOR HEREBY AGREES (a) to communicate to ASSIGNEE, its successors and assigns, or their representatives or agents, all facts and information known or available to ASSIGNOR respecting said invention or inventions, improvements, and modifications including evidence for interference, reexamination, reissue, opposition, revocation, extension, or infringement purposes or other legal, judicial, or administrative proceedings, whenever requested by ASSIGNEE; (b) to testify in person or by affidavit as required by ASSIGNEE, its successors and assigns, in any such proceeding in the United States or a country foreign thereto; (c) to execute and deliver, upon request by ASSIGNEE, all lawful papers including, but not limited to, original, divisional, continuation, and reissue applications, renewals, assignments, powers of attorney, oaths, affidavits, declarations, depositions; and (d) to provide all reasonable assistance to ASSIGNEE, its successors and assigns, in obtaining and enforcing proper title in and protection for said invention or inventions, improvements, and modifications under the intellectual property laws of the United States and countries foreign thereto.

ASSIGNOR HEREBY REPRESENTS AND WARRANTS that ASSIGNOR has the full and unencumbered right to sell, assign, and transfer the interests sold, assigned, and transferred herein, and that ASSIGNOR has not executed and will not execute any document or instrument in conflict herewith.

ASSIGNOR HEREBY GRANTS to the law firm of **Foley & Lardner** the power and authority to insert in this Assignment any further identification which may be necessary or desirable to comply with the rules of the U.S. Patent and Trademark Office for recordation of this Assignment.

ASSIGNOR UNDERSTANDS AND AGREES that the attorneys and agents of the law firm of **Foley & Lardner** do not personally represent ASSIGNOR or ASSIGNOR's legal interests, but instead represent the interests of ASSIGNEE; since said attorneys and agents cannot provide legal advice to ASSIGNOR with respect to this Assignment, ASSIGNOR acknowledges its right to seek its own independent legal counsel.

Executed this 19 day of February, 2004

VLADIMIR SABETSKY

State of DISTRICT OF COLUMBIA)
) ss.
County of _____)

On this 19TH day of FEBRUARY, 2004, before me, a notary public in and for said county, appeared Vladimir SABETSKY, who is personally known to me to be the same person whose name is subscribed to the foregoing instrument, and he/she acknowledged that he/she signed, sealed, and delivered the said instrument as his/her free and voluntary act for the uses and purposes therein set forth.

Notary Public [illegible]

My Commission Expires: _____

(Seal)

My Commission Expires
August 14, 2008

.1011110.1

ASSIGNMENT AND AGREEMENT

WHEREAS, Vladimir SABETSKY of Piskarevsky Prospekt 10, q.320, 105221 Saint-Petersburg, Russia; (hereinafter referred to singly and collectively as "ASSIGNOR") have invented a certain invention entitled NOVEL DELIVERY SYSTEM FOR DRUG AND CELL THERAPY (Atty. Dkt. No. 028093-0103) for which a provisional application for United States Letters Patent was filed on May 5, 2003 as Application No. 60/467,601; and

WHEREAS, THE TECHNOLOGY DEVELOPMENT COMPANY Ltd., a Bermuda corporation (hereinafter referred to as "ASSIGNEE") is desirous of acquiring the entire interest therein;

NOW THEREFORE, in consideration of One Dollar ($1.00) and other good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged, ASSIGNOR has sold, assigned, and transferred, and by these presents hereby sells, assigns, and transfers, unto ASSIGNEE, its successors and assigns, the full and exclusive right, title, and interest in and to (a) the above-identified invention or inventions and all improvements and modifications thereof, (b) the above-identified application and all other applications for Letters Patent of the United States and countries foreign thereto for the above-identified invention or inventions and all improvements and modifications thereof, (c) all Letters Patent which may issue from said applications in the United States and countries foreign thereto, (d) all divisions, continuations, reissues, and extensions of said applications and Letters Patent, and (e) the right to claim for any of said applications the full benefits and priority rights under the International Convention and any other international agreement to which the United States adheres; such right, title, and interest to be held and enjoyed by ASSIGNEE, its successors and assigns, to the full end of the term or terms for which any and all such Letters Patent may be granted as fully and entirely as would have been held and enjoyed by ASSIGNOR had this Assignment not been made.

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VLADIMIR SABETSKY

State of DISTRICT OF COLUMBIA)
) ss.
County of _____)

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Notary Public Wendy Hudson

My Commission Expires: _____

(Seal)

My Commission Expires
August 14, 2008

345
348

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WHEREAS, Vladimir SABETSKY of Piskarevsky Prospekt 10, q.320, 105221 Saint-Petersburg, Russia; (hereinafter referred to singly and collectively as "ASSIGNOR") have invented a certain invention entitled NOVEL DELIVERY SYSTEM FOR DRUG AND CELL THERAPY (Atty. Dkt. No. 028093-0105) for which a provisional application for United States Letters Patent was filed on May 9, 2003 as Application No. 60/469,017; and

WHEREAS, THE TECHNOLOGY DEVELOPMENT COMPANY Ltd., a Bermuda corporation (hereinafter referred to as "ASSIGNEE") is desirous of acquiring the entire interest therein;

NOW THEREFORE, in consideration of One Dollar ($1.00) and other good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged, ASSIGNOR has sold, assigned, and transferred, and by these presents hereby sells, assigns, and transfers, unto ASSIGNEE, its successors and assigns, the full and exclusive right, title, and interest in and to (a) the above-identified invention or inventions and all improvements and modifications thereof, (b) the above-identified application and all other applications for Letters Patent of the United States and countries foreign thereto for the above-identified invention or inventions and all improvements and modifications thereof, (c) all Letters Patent which may issue from said applications in the United States and countries foreign thereto, (d) all divisions, continuations, reissues, and extensions of said applications and Letters Patent, and (e) the right to claim for any of said applications the full benefits and priority rights under the International Convention and any other international agreement to which the United States adheres; such right, title, and interest to be held and enjoyed by ASSIGNEE, its successors and assigns, to the full end of the term or terms for which any and all such Letters Patent may be granted as fully and entirely as would have been held and enjoyed by ASSIGNOR had this Assignment not been made.

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ss.
County of _____)

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Notary Public WENDY HUBBARD

My Commission Expires: _____

(Seal)

My Commission Expires
August 14, 2008

350
350

ASSIGNMENT AND AGREEMENT

WHEREAS, Vladimir SABETSKY of Piskarevsky Prospekt 10, q.320, 105221 Saint-Petersburg, Russia; (hereinafter referred to singly and collectively as "ASSIGNOR") have invented a certain invention entitled **NOVEL DELIVERY SYSTEM FOR DRUG AND CELL THERAPY** (Atty. Dkt. No. 028093-0106) for which a provisional application for United States Letters Patent was filed on August 15, 2003 as Application No. 60/495,097; and

WHEREAS, THE TECHNOLOGY DEVELOPMENT COMPANY Ltd., a Bermuda corporation (hereinafter referred to as "ASSIGNEE") is desirous of acquiring the entire interest therein;

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State of DISTRICT OF COLUMBIA)
) ss.
County of _____)

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Notary Public WENDY HUSBAND

My Commission Expires: _____

(Seal)

My Commission Expires
August 14, 2008

Industria y Comercio

FORMATO DE DIGITALIZACION
RELACION DE ANEXOS

Royal Technologies S.A.

352

Item		Expediente No	05100645	No de Folio
			No de Unidades	√
1	CD			
2	DVD			
3	REVISTA			
4	LIBRO			
5	PERIODICO			
6	DISQUETES			
7	SOBRE DE MANILA	X	1	
8	SOBRE BLANCO TAMAÑO CARTA			
9	SOBRE BLANCO TAMAÑO OFICIO			
10	OTROS:			

Observaciones: Sobre Con Documentos Alta Final

353

379

SUPERINTENDENCIA DE COMERCIO
Radicacion : 05100615 00000000 Folios: 352
Fecha (AMD): 2005-10-04 17:00:29

Tramite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS SOLICITUDES

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCIÓN PCT MODELO DE UTILIDAD	( )	( )
- Indicación de que se solicita la concesión de una patente.	( )	X
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	( )	X
- Descripción de la Invención.	( )	X
- Dibujos de ser estos pertinentes.	( )	X
- Comprobante de Pago de las tasas establecidas.		
COMPLETA (X)	INCOMPLETA ( )	( )
DISEÑO INDUSTRIAL ( )		
- Indicación de que se solicita el registro de Diseño Industrial	( )	( )
- Datos de identificación del solicitante o de la persona que presenta	( )	( )
- la solicitud.		
- Representación gráfica o fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.	( )	( )
Comprobante de pago de las tasas establecidas	( )	( )
COMPLETA ( )	INCOMPLETA ( )	( )
ESQUEMA DE TRAZADO ( )		
- Indicación de que solicita el registro de un Esquema de trazado.	( )	( )
- Datos de identificación del solcitante o de la persona que presenta La solicitud.	( )	( )
Representación gráfica del esquema de trazado	( )	( )
Comprobante de pago de las tasas establecidas.	( )	( )
COMPLETA ( )	INCOMPLETA ( )	( )

Firma Responsable:

Fecha:

354 354

REPUBLICA DE COLOMBIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
RODRIGUEZ D'ALEMAN DILIA MARIA

26604

REFERENCIA	Radicación No.	5 100645
	Solicitud internacional	IB2004/000961
	Fecha inicio fase nacional	04/10/2005
	Trámite	11
	Evento	379
	Actuación	430
	Oficio No.	13600

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. El solicitante deberá presentar el documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante, debidamente autenticada y apostillada.

2. La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.

CLARA DE JAIMES
Jefe de la división de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista de fecha 29 DIC. 2005
adpa11