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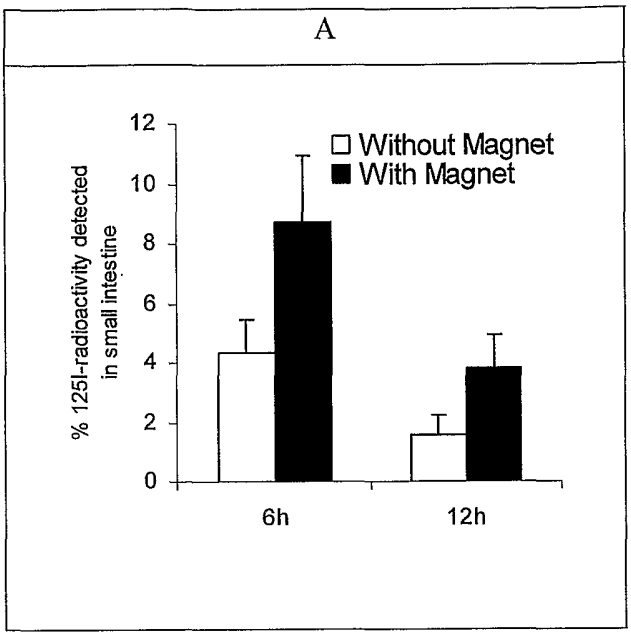
(54) Title: MAGNETICALLY-LABELED MICROPARTICLES FOR ORAL DRUG DELIVERY



(57) Abstract: A pharmaceutically active agent is associated with a magnetic particle and formulated for oral administration. An externally imposed magnetic field retains the magnetic particle in the intestines, increasing the amount of the pharmaceutically active agent that passes from the intestines into the bloodstream.

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Magnetically-labeled Microparticles for Oral Drug Delivery

Field of the Invention

This invention relates to the use of magnetic particles to enhance the bioavailability of orally administered pharmaceuticals.

Background of the Invention

Oral delivery of peptides and protein therapeutics has been extensively studied in the past several decades. This route of administration is preferred because it increases patient compliance and comfort compared to the parenteral route, which accounts for the administration of more than ninety percent of FDA approved protein drugs. However, clinically effective oral delivery systems for protein therapeutics have not been established [1].

Proteins administered orally result in extremely poor absorption into the circulatory system due to the degradation of proteins in harsh acidic and enzymatic conditions in the stomach, and low permeation of proteins across the intestinal membranes [1]. To alleviate these difficulties, many approaches have been used for protecting proteins, such as insulin, from degradation during administration. These approaches have included encapsulation within pH-sensitive hydrogels (2), liposomes (3-5), polymeric nanoparticles (6, 7), and the use of permeation enhancers (8-10) and enzyme inhibitors (11).

Polymeric microparticles (MPs) are easy to prepare, encapsulate protein with high efficiency (usually greater than 50%), and effectively protect encapsulated proteins from degradation in gastrointestinal tract (GIT) [1]. A drawback of this approach is that many of the particles pass through the small intestines without being absorbed. Retention of these protein-containing particles in the small intestine for an extended period of time may result in an increase of the delivery efficiency through either the absorption of localized particles or through the absorption of protein drugs that are released in small intestine from these particles. Different approaches have been attempted to slow down the intestinal transit of orally administrated drug carriers. For example, mucoadhesive polymeric particles that can adhere to mucus layer in the intestine have been studied [12].

Magnetically modulated particulate systems have attracted much attention recently for use in in vivo imaging and in targeted drug delivery. Imaging ligands or drugs can be readily localized at targeted sites through an external magnetic field. As a result, it would be desirable to have magnetic particulate carriers that may be localized by an external magnetic field in intestinal areas for effective oral delivery of protein [13].

Definitions

"**Bioavailability**": The term "bioavailability", as used herein, refers to the rate at which and extent to which an active agent is absorbed or is otherwise available to a treatment site in the body. For active agents that are encapsulated in a biodegradable polymer or pharmaceutically acceptable carrier, or both, bioavailability also depends on the extent to which the active agent is released from the polymer and/or carrier into the bloodstream.

"**Biomolecules**": The term "biomolecules", as used herein, refers to molecules (e.g., proteins, amino acids, peptides, polynucleotides, nucleotides, carbohydrates, sugars, lipids, nucleoproteins, glycoproteins, lipoproteins, steroids, etc.) whether naturally-occurring or artificially created (e.g., by synthetic or recombinant methods) that are commonly found in cells and tissues. Specific classes of biomolecules include, but are not limited to, enzymes, receptors, neurotransmitters, hormones, cytokines, cell response modifiers such as growth factors and chemotactic factors, antibodies, vaccines, haptens, toxins, interferons, ribozymes, anti-sense agents, plasmids, DNA, and RNA.

"**Biocompatible**": The term "biocompatible", as used herein is intended to describe compounds that are not toxic to cells. Compounds are "biocompatible" if their addition to cells in vitro results in less than or equal to 20 % cell death, and their administration in vivo does not induce significant inflammation or other such significant adverse effects.

"**Biodegradable**": As used herein, "biodegradable" polymers are polymers that degrade fully (i.e., down to monomeric species) under physiological or endosomal conditions. In preferred embodiments, the polymers and polymer biodegradation byproducts are biocompatible. Biodegradable polymers are not

necessarily hydrolytically degradable and may require enzymatic action to fully degrade.

"**Endosomal** conditions": The phrase "endosomal conditions", as used herein, relates to the range of chemical (e.g., pH, ionic strength) and biochemical (e.g., enzyme concentrations) conditions likely to be encountered within endosomal vesicles. For most endosomal vesicles, the endosomal pH ranges from about 5.0 to 6.5.

"**Physiological conditions**": The phrase "physiological conditions", as used herein, relates to the range of chemical (e.g., pH, ionic strength) and biochemical (e.g., enzyme concentrations) conditions likely to be encountered in the intracellular and extracellular fluids of tissues. For most tissues, the physiological pH ranges from about 7.0 to 7.4.

"**Polynucleotide**", "**nucleic acid**", or "**oligonucleotide**": The terms "polynucleotide", "nucleic acid", or "oligonucleotide" refer to a polymer of nucleotides. The terms "polynucleotide", "nucleic acid", and "oligonucleotide", may be used interchangeably. Typically, a polynucleotide comprises at least two nucleotides. DNAs and RNAs are polynucleotides. The polymer may include natural nucleosides (i.e., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine), nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, C5-propynylcytidine, C5-propynyluridine, C5 bromouridine, C5 fluorouridine, C5 iodouridine, C5 methylcytidine, 7 deazaadenosine, 7 deazaguanosine, 8 oxoadenosine, 8 oxoguanosine, O(6) methylguanine, and 2-thiocytidine), chemically modified bases, biologically modified bases (e.g., methylated bases), intercalated bases, modified sugars (e.g., 2′-fluororibose, ribose, 2′-deoxyribose, arabinose, and hexose), or modified phosphate groups (e.g., phosphorothioates and 5′-N phosphoramidite linkages). Enantiomers of natural or modified nucleosides may also be used. Nucleic acids also include nucleic acid-based therapeutic agents, for example, nucleic acid ligands, siRNA, short hairpin RNA, antisense oligonucleotides, ribozymes, aptamers, and SPIEGELMERS™, oligonucleotide ligands described in Wlotzka, et al., Proc. Nat'l. Acad. Sci. USA, 2002, 99(13):8898, the entire contents of which are incorporated herein by reference.

"**Polypeptide**", "**peptide**", or "**protein**": According to the present invention, a "polypeptide", "peptide", or "protein" comprises a string of at least three amino acids linked together by peptide bonds. The terms "polypeptide", "peptide", and "protein", may be used interchangeably. Peptide may refer to an individual peptide or a collection of peptides. Inventive peptides preferably contain only natural amino acids, although non natural amino acids (i.e., compounds that do not occur in nature but that can be incorporated into a polypeptide chain) and/or amino acid analogs as are known in the art may alternatively be employed. Also, one or more of the amino acids in a peptide may be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modification, etc. In one embodiment, the modifications of the peptide lead to a more stable peptide (e.g., greater half-life in vivo). These modifications may include cyclization of the peptide, the incorporation of D-amino acids, etc. None of the modifications should substantially interfere with the desired biological activity of the peptide.

"**Polysaccharide**", "**carbohydrate**" or "**oligosaccharide**": The terms "polysaccharide", "carbohydrate", or "oligosaccharide" refer to a polymer of sugars. The terms "polysaccharide", "carbohydrate", and "oligosaccharide", may be used interchangeably. Typically, a polysaccharide comprises at least two sugars. The polymer may include natural sugars (e.g., glucose, fructose, galactose, mannose, arabinose, ribose, and xylose) and/or modified sugars (e.g., 2′-fluororibose, 2′-deoxyribose, and hexose).

"**Small molecule**": As used herein, the term "small molecule" is used to refer to molecules, whether naturally-occurring or artificially created (*e.g.*, via chemical synthesis) that have a relatively low molecular weight. In some embodiments, small molecules are monomeric and have a molecular weight of less than about 1500 g/mol. Exemplary small molecules are biologically active in that they produce a local or systemic effect in animals, preferably mammals, more preferably humans. Small molecules include, but are not limited to, radionuclides and imaging agents, for example, for magnetic resonance imaging (MRI), computer aided tomography (CAT scans), X-ray contrast agents, fluorescence contrast agents, and other imaging agents used for medical diagnosis. In certain embodiments, the small molecule is a drug.

Preferably, though not necessarily, the drug is one that has already been deemed safe and effective for use by the appropriate governmental agency or body. For example, drugs for human use listed by the FDA under 21 C.F.R. §§ 330.5, 331 through 361, and 440 through 460; drugs for veterinary use listed by the FDA under 21 C.F.R. §§ 500 through 589, incorporated herein by reference, are all considered acceptable for use in accordance with the present invention. Known naturally-occurring small molecules include, but are not limited to, penicillin, erythromycin, taxol, cyclosporin, and rapamycin. Known synthetic small molecules include, but are not limited to, ampicillin, methicillin, sulfamethoxazole, and sulfonamides.

"Bioactive agents": As used herein, "bioactive agents" is used to refer to compounds or entities that alter, inhibit, activate, or otherwise affect biological or chemical events. For example, bioactive agents may include, but are not limited to, anti-AIDS substances, anti-cancer substances, antibiotics, immunosuppressants, anti-viral substances, enzyme inhibitors, including but not limited to protease and reverse transcriptase inhibitors, fusion inhibitors, neurotoxins, opioids, hypnotics, anti-histamines, lubricants, tranquilizers, anti-convulsants, muscle relaxants and anti-Parkinson substances, anti-spasmodics and muscle contractants including channel blockers, miotics and anti-cholinergics, anti-glaucoma compounds, anti-parasite and/or anti-protozoal compounds, modulators of cell-extracellular matrix interactions including cell growth inhibitors and anti-adhesion molecules, vasodilating agents, inhibitors of DNA, RNA or protein synthesis, anti-hypertensives, analgesics, anti-pyretics, steroidal and non-steroidal anti-inflammatory agents, anti-angiogenic factors, anti-secretory factors, anticoagulants and/or antithrombotic agents, local anesthetics, ophthalmics, prostaglandins, anti-depressants, anti-psychotic substances, anti-emetics, and imaging agents. In a certain embodiments, the bioactive agent is a drug.

A more complete listing of bioactive agents and specific drugs suitable for use in the present invention may be found in "Pharmaceutical Substances: Syntheses, Patents, Applications" by Axel Kleemann and Jurgen Engel, Thieme Medical Publishing, 1999; the "Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals", Edited by Susan Budavari *et al.*, CRC Press, 1996, and the United States Pharmacopeia-25/National Formulary-20, published by the United States

Pharmcopeial Convention, Inc., Rockville MD, 2001, all of which are incorporated herein by reference.

As used herein, the term "**pharmaceutically active agent**" refers collectively to biomolecules, small molecules, and bioactive agents.

Summary of the Invention

In one aspect, the invention is a drug delivery composition comprising a magnetic particle, a pharmaceutically active agent associated with the particle, and an ingestible carrier containing the particle. The magnetic particle may have a dimension between 5 nm and 15 mm, for example, greater than 5 microns. The magnetic particle may be functionalized with one or more of biotin, streptavidin, protein A, protein G, an oligonucleotide, an amine, a carboxylate, and an organosilane. The pharmaceutically active agent may be associated with the particle by a covalent or a non-covalent interaction. The composition may further include a plurality of magnetic particles, wherein a biologically active agent is associated with each particle. For example, the magnetic particle and the pharmaceutically active agent may be co-encapsulated within a polymer particle. Alternatively or in addition, the pharmaceutically active agent may be encapsulated in polymeric particles, and the magnetic particle may be associated with at least one of the polymeric particles. The polymeric particles may include a synthetic polymer or a non-synthetic polymer. The composition may include a plurality of polymeric particles associated with at least one magnetic particle or with a plurality of magnetic particles in an extended network. The pharmaceutically active agent may be associated with a polymer, and the polymer may be associated with the magnetic particle.

In another aspect, the invention is a method of delivering a pharmaceutically active agent. The method includes providing the agent associated with a magnetic particle and orally administering the agent to a patient in need thereof. The method may further include applying a magnetic field to at least a portion of the lower gastrointestinal tract of the patient. The magnetic particle may pass through the intestinal wall to deliver the pharmaceutically active agent to the bloodstream. The magnetic particle may retain the pharmaceutically active agent in the intestine until it is able to pass through the intestinal wall into the bloodstream, while the magnetic particle does not pass through the intestinal wall. The method may further include

using a magnet to retain the magnetic particles at a tissue site that is not a target tissue site of the pharmaceutically active agent. The magnet or a plurality of magnets may be disposed on a garment such as a belt.

In another aspect, the invention is a method of preparing a pharmaceutical composition. The method includes associating a pharmaceutically active agent with a polymer to form an agent-polymer aggregate and associating the agent-polymer aggregate with a magnetic particle. The agent-polymer aggregate may have a predetermined surface charge, and one or more agent-polymer aggregates may be associated with the magnetic particle via a charge interaction. Associating the agent-polymer aggregate may include encapsulating the agent and at least one a magnetic particle in the polymer.

In another aspect, the invention is a kit for delivering a pharmaceutically active agent. The kit includes a pharmaceutically acceptable carrier containing a plurality of magnetic particles having the agent associated with them and a magnet sufficiently strong to hinder the passage of the particles away from a tissue site when the magnet is placed in the vicinity of the tissue site.

In another aspect, the invention is a composition including a plurality of magnetic particles, a pharmaceutically active agent associated with the particles, and an ingestible carrier within which the plurality of magnetic particles are distributed.

Brief Description of the Drawing

The invention is described with reference to the several figures of the drawing, in which,

Fig. 1. SEM image of Humulin R-encapsulated, magnetite PLGA MP. Magnetite content in weight percentage: A) 0%, B) 2%, C) 5%, D) 10%.

Fig. 2. Homogenized small intestine solution of mice in the group of A) no administration of magnetic particle; B) receiving 5 mg of fluorescent YG superparamagnetic microspheres and being restrained for 6h in the absence of external magnet; C) receiving 5 mg of fluorescent YG superparamagnetic microspheres and being restrained for 6h in the presence of external magnet.

Fig. 3. A) Mice treated with 1 μCi 125I-insulin-magnetite (2 wt%)-PLGA microparticles, restrained in the presence or absence of a magnetic field. Small intestines were collected, solubilized and analyzed using scintillation counter at 6 h

and 12 h. B) Glucose reduction by Humulin R-magnetite-PLGA microparticles (50 unit/kg) in the presence and absence of magnetic field.

Fig. 4. Particle complex formed through the interaction of negatively charged Dextran-Rhodamine-PLA-COOH MP and positively charged BioMag-NH_2 particles (average size 6 μm) A) fluorescence image of complex taken using 20× objective and rhodamine filter; B) light optical image of the complex. C) overlay of the image A and B.

Fig. 5. SEM images: PLA-COOH-MP/BioMag-NH_2 (6 μm) complex via charge interaction

Fig. 6. SEM images: Interaction of MP and BioMag Plus (1 μm) through charge interaction. A) PLA-COOH MP; B) BioMag-NH2 (1 μm); C) BioMag-COOH (1 μm); D) BioMag COOH/PLA-Chitosan MP complex; E) BioMag NH_2/PLA-COOH MP complex; F) BioMag COOH/ PLA-COOH MP complex

Fig. 7. Schematic representation of the *in vitro* flow apparatus for the study of magnetic responsiveness of the ^{125}I insulin-magnetite-PLGA microparticles. Microparticles containing 0.5 μCi ^{125}I radioactivity were introduced to the flow system through syringe A. Syringe B delivered a mobile phase at 2.5 mL/min (PBS, 1x, pH = 7.4). A magnet was placed under the silicone tubing between the injection site and the collection vials.

Fig. 8. Magnetic response of BioMag Plus-NH_2/^{125}I-Insulin PLGA MP complex: cumulative elution profile from the in vitro flow apparatus with and without the effect of magnetic field.

Fig 9. Small intestine of mice administered with PLA-COOH MP/BioMag-NH_2 and applied with (left) and without magnet (right).

Fig. 10. Histology analysis of mice intestine 8 h after administration of BioMag-NH2/Dextran-Rhodamine/PLA-COOH MP and BioMag-COOH/Chitosan-coated Dextran-Rhodamine-PLA-COOH MP. A) BioMag-COOH/Chitosan-coated Rhodamine-Dextran-PLA-COOH MP without magnet; B) BioMag-NH2/Rhodamine-Dextran-PLA-COOH MP without magnet; C-D) BioMag-NH2/Rhodamine-Dextran-PLA-COOH MP with magnet; E-F) BioMag-COOH/Chitosan-coated Rhodamine-Dextran-PLA-COOH MP with magnet.

Fig. 11. Recovered total radioactivity in small intestine. Mice were

administered 1 μCi eq. BioMag-NH2 (6 μm)/^{125}I-Insulin-PLA-COOH MP complex and euthanized 19 h after administration. Small intestine and blood were collected, dissolved by Solvable (Perkin Elmer) and analyzed on a liquid scintillation analyzer.

Fig. 12. Glucose reduction study using the complex of BioMag-NH2/Humulin R-PLGA-COOH NPs or MPs with and without external magnet.

Fig. 13A. Elution of ^{125}I-insulin-PLGA MPs containing 0%, 2% and 5% magnetite from the *in vitro* flow apparatus (shown in Figure 2) in the presence of a magnetic field.

Fig. 13B. Cumulative profile for the elution of ^{125}I-insulin-PLGA MPs containing 0%, 2% and 5% magnetite from the in vitro flow apparatus (shown in Figure 2) in the presence of a magnetic field.

Fig. 14. Glucose reduction by insulin (0.5 U/kg, i.v.) and by insulin-magnetite (8%)-PLGA microparticles (100 U/kg) in mice in the presence or absence of a magnetic field ($P_{w\text{-}w/o} < 0.01$).

Fig. 15. Serum insulin concentration of oral administration of insulin-magnetite (8%)-PGLA microparticles (100 U/kg) in mice in the presence or absence of a magnetic field ($p_{w\text{-}w/o}<0.03$) (A). Serum insulin concentration of tail vein i.v. administration of insulin (2 U/kg) in mice (B).

Fig. 16. H&E-stained tissue sections magnified in 50x from the organs: (A) small intestine, (B) liver, (C) spleen, (D) kidney. Images on the left show sections from controls, whereas images on the right were taken from mice administered with magnetic encapsulated microparticles.

Fig. 17. Colloidal iron (Mallory method) stained sections magnified 50x from the organs: (A) small intestine and (B) liver. Left images are control, and right images are magnetite-treated mice. Inset in (A) is positive control for stained magnetites.

Fig. 18. Schematic illustration of particle complexes formed through the interaction of negatively charged biopharmaceutical encapsulated polymeric microparticles (PolyMP-) and positively charged magnetic microparticles (MagMP+).

Fig. 19. Complexes formation due to charge interaction of positively charged MagMP+ or negatively charged MagMP- with negatively charged PolyMP-: PolyMP- alone (up-left); MagMP+ alone (up-right); MagMP+/PolyMP- complex with external magnetic field, immediate precipitation of both MagMP+ and PolyMP- was observed

when a magnet was applied (low-left); MagMP-/PolyMP- complex with external magnetic field, immediate precipitation of only MagMP-, but not PolyMP-, was observed when an magnet was applied (low-right).

Fig. 20. Complexes of ^{125}I-insulin encapsulated PolyMP- and MagMP+ with various mass ratios.

Fig. 21. Stability of the complex of ^{125}I-insulin encapsulated PolyMP- and MagMP+ (mass ratio = 1/1) at various pH.

Fig. 22. Cumulative elution profile from an *in vitro*, horizontal flow apparatus with and without the effect of magnetic field for the study of magnetic responsiveness of ^{125}I -Insulin encapsulated PolyMP-/MagMP+ complexes. The strength of the coupling and the retentive force of the particles in the intestine by placement of a small neodymium (NdFeB) plate magnet near the abdomen were examined by administering ^{125}I-insulin in coupled PLA particles to balb/c mice. Recovered total radioactivity in small intestine of mice were administered 1 μCi eq. ^{125}I-Insulin encapsulated PolyMP-/MagMP+. Small intestine and blood were collected 6h after dosing, dissolved by Solvable (Perkin Elmer) and analyzed on a liquid scintillation analyzer ($P < 0.05$). After 8 hours, the biodistribution of the ^{125}I -insulin was determined for organs in the animals. 32.5% of the insulin was recovered still in the small intestine compared with 5.2% for the control animals. Further, 86% more ^{125}I-insulin was recovered in the blood of the magnet-applied group compared to control.

Fig. 23 Histology of the small intestine of mice orally dosed with the complexes of rhodamine-dextran encapsulated PolyMP- and MagMP+ in the absence (A) and presence (B) of an abdominally applied magnet for 8 hours. Images are overlays showing fluorescence of retained particles in small intestine.

Fig. 24 Glucose reduction assay in mice (n = 4) with the complex of Humulin R encapsulated PolyMag- and MagMP+ in the presence and absence of external magnet ($P < 0.05$). Bioavaliabilities (*f*) calculated on area_under_curve (AUC): $f = 5 \pm 1.24$ and 0.82 ± 0.37 in the presence and absence of abdominally applied magnetic field, respectively (a); Heparin concentration detected in the blood of treated mice (n = 4) with the complex of heparin encapsulated PolyMP– and MagMP+ in the presence and absence of abdominally applied magnetic field ($P < 0.01$). Bioavailablities $f = 4.05 \pm 0.16$ in the presence of magnetic field and 2.89 ± 0.32 in the absence of magnetic field (b); Representative tissue sections magnified in 50X

from the small intestine. The images on c1 show sections from no treatment whereas the images on c2 were taken from mice administered with PolyMP-/MagMP + with an applied magnetic field for 24h in the abdominal area of mice.

Detailed Description of Certain Preferred Embodiments

By associating a pharmaceutically active agent with a magnetic particle, we have developed a delivery vehicle that increases the bioavailability of the agent and the ease of delivery to a patient. The magnetic particle allows even protein and nucleic acid-based agents to be delivered orally. Orally administered agents overcome numerous obstacles before finally entering the bloodstream. Chemical obstacles include the extreme pH environment of the stomach (1.2-3), the broad pH range of the intestine (ranging from about 6 in the duodenum to about 8 in the jejunum and ilium), and the digestive enzymes, such as pepsin and trypsin, that are found throughout the digestive tract. In addition, pharmaceutically active agents must traverse a mucus layer and pass through or between the epithelial cells lining the intestines to reach the bloodstream from the digestive tract.

In one embodiment, the pharmaceutically active agent is associated with the magnetic particle. The pharmaceutically active agent may be covalently or non-covalently linked to the particle or may be covalently or non-covalently linked to a material that is in turn covalently or non-covalently linked to the magnetic particle. Alternatively or in addition, the pharmaceutically active agent may be co-encapsulated or otherwise physically associated with the magnetic particle.

The active agents to be incorporated in the controlled release polymer system of the present invention may be therapeutic, diagnostic, prophylactic or prognostic agents. Any chemical compound to be administered to an individual may be delivered using the conjugates of the invention. The active agent may be a small molecule, organometallic compound, nucleic acid, protein, peptide, metal, an isotopically labeled chemical compound, drug, vaccine, immunological agent, etc. Exemplary active agents include small molecules, biomolecules, and bioactive agents as defined herein. The teachings of the invention may be particularly useful for pharmaceutical agents that have limited bioavailability when administered orally. For example, protein and antibody-based drugs are difficult to administer orally and are often administered intravenously or subcutaneously, via injection. Insulin is the most

common example of these agents. Other agents that will especially benefit from the teachings of the invention include nucleic acid based drugs such as whole genes, anti-sense agents, and RNAi agents. Some small molecule drugs are also difficult to formulate for oral delivery. Because the magnetic particles help retain the pharmaceutically active agent in the small intestine, they can help slow the penetration of highly permeable drugs through the intestinal wall and help retain drug delivery vehicles in the intestine that would otherwise pass entirely through the digestive tract and be eliminated within a few hours.

In one embodiment, the agents are organic compounds with pharmaceutical activity. In another embodiment of the invention, the agent is a small molecule that is a clinically used drug. In exemplary embodiments, the drug is an antibiotic, anti-viral agent, anesthetic, steroidal agent, anti-inflammatory agent, anti-neoplastic agent, antigen, vaccine, antibody, decongestant, antihypertensive, sedative, birth control agent, progestational agent, anti-cholinergic, analgesic, anti-depressant, anti-psychotic, adrenergic blocking agent, diuretic, cardiovascular active agent, vasoactive agent, non-steroidal anti-inflammatory agent, nutritional agent, etc. While many small molecule drugs are already available for oral administration, some are not sufficiently soluble to be orally administered and may benefit from the techniques described herein.

In another embodiment, the agent is a protein drug, such as an antibody, an antibody fragment, a recombinant antibody, a recombinant protein, a purified protein, a peptide, an amino acid and combinations thereof. Exemplary protein drugs include but are not limited to biologically active macromolecules such as enzyme inhibitors, colony-stimulating factors, plasminogen activators, polypeptide hormones, insulin, myelin basic protein, collagen S antigen, calcitonin, angiotensin, vasopressin, desmopressin, LH-RH (luteinizing hormone-releasing hormone), somatostatin, glucagon, somatomedin, oxytocin, gastrin, secretin, h-ANP (human atrial natriuretic polypeptide), ACTH (adrenocorticotropic hormone), MSH (melanocyte stimulating hormone), beta-endorphin, muramyl dipeptide, enkephalin, neurotensin, bombesin, VIP (vasoactive intestinal peptide), CCK-8 (cholecystokinin), PTH (parathyroid hormone), CGRP (calcitonin gene related peptide), endothelin, TRH (thyroid releasing hormone), interferons, cytokines, streptokinase, urokinase, and growth factors. Exemplary growth factors include but are not limited to activin A (ACT).

retinoic acid (RA), epidermal growth factor, bone morphogenetic protein, platelet derived growth factor, hepatocyte growth factor, insulin-like growth factors (IGF) I and II, hematopoietic growth factors, peptide growth factors, erythropoietin, angiogenic factors, anti-angiogenic factors, interleukins, tumor necrosis factors, interferons, colony stimulating factors, t-PA (tissue plasminogen activator), G-CSF (granulocyte colony stimulating factor), heparin binding growth factor (HBGF), alpha or beta transforming growth factor (α- or β-TGF), fibroblastic growth factors, epidermal growth factor (EGF), vascular endothelium growth factor (VEGF), nerve growth factor (NGF) and muscle morphogenic factor (MMP). Also suitable for use with the invention are recombinantly-produced derivatives of therapeutically useful proteins, including deletion, insertion and substitution variants, which on the whole have similar or comparable pharmacological properties.

Gene therapy technology may also benefit from the techniques of the invention. Genetic material is typically not stable in the GI tract. Polymer encapsulation can protect genetic material and "escort" it through the GI tract and into the bloodstream. In one embodiment, the active agent delivered using the techniques of the invention is a nucleic acid based drug, such as DNA, RNA, modified DNA, modified RNA, antisense oligonucleotides, expression plasmid systems, nucleotides, modified nucleotides, nucleosides, modified nucleosides, nucleic acid ligands (e.g. aptamers), intact genes, a promoter complementary region, a repressor complementary region, an enhancer complementary region, and combinations thereof. A promoter complementary region, a repressor complementary region, or an enhancer complementary region can be fully complementary or partially complementary to the DNA promoter region, repressor region, an enhancer region of a gene for which it is desirable to modulate expression. For example, it may be at least 50% complementary, at least 60% complementary, at least 70% complementary, at least 80% complementary, at least 90% complementary, or at least 95% complementary.

Genetic material is acidic and will form electrostatic bonds with cationic polymers. If it is desirable to avoid strong ionic interactions, nucleic acid based drugs can be encapsulated with anionic polymers or other hydrophilic polymers that do not have cationic groups. For example, polymers modified with short poly(cytosine) tags may be used to encapsulate genetic material. Other examples include but are not limited to polysebacic anhydride (PSA) and poly(lactic acid). These polymers may be

modified to carry a more negative charge, for example, a terminal carboxylic acid group can be added to poly(lactic acid).

In another embodiment, the controlled release polymer systems may deliver a diagnostic or prognostic agent used for long term diagnosis of a patient's health. For example, kidney function is determined by delivering an agent, such as creatinine, to the bloodstream that is cleared solely by the glomerulus and then measuring the concentration of the agent in the blood or urine over time. The controlled release particles of the invention can be used to provide a steady state concentration of the clearance agent in the bloodstream for an extended period of time, and periodic assays of the concentration of the agent in the patient's urine can be used to determine the rate of clearance of the agent by the kidneys. Alternative clearance agents, for example, agents that are cleared from the body through other mechanisms, e.g, by the liver or through other metabolic processes, may also be encapsulated and delivered using the controlled release polymer systems described herein.

Prophylactic agents that can be delivered to a patient by exploiting the invention include, but are not limited to, antibiotics and nutritional supplements. For example, the techniques of the invention may be used to deliver nutrients to patients experiencing a deficiency or who are unable to produce or store such substances themselves. For example, vitamin D may be delivered to patients who are unable to synthesize it.

Vaccines and antigens are additional prophylactic agents that may be administered to a patient using the techniques of the invention. Some vaccines require extended exposure to the immune system to stimulate the desired immune response. Micro- or nanoparticles containing a vaccine or antigen may be suspended in a fluid or charged into a capsule and ingested, allowing patients to receive their vaccine orally instead of as an injection. A single administration of a dose of particles produced according to the invention may substitute for multiple injections or reduce the number of administrations. Of course, fast-decomposing particles may be fabricated to encapsulate vaccines that do not require extended exposure. Formulation of the vaccine as a capsule, pill, or ingestible liquid may also improve the shelf life of the vaccine, easing delivery of vaccines to rural or impoverished areas.

Vaccines may comprise isolated proteins or peptides, inactivated organisms and viruses, dead organisms and viruses, genetically altered organisms or viruses, and

cell extracts. Prophylactic agents may be combined with interleukins, interferon, cytokines, and adjuvants such as cholera toxin, alum, Freund's adjuvant, etc. Prophylactic agents include antigens of such bacterial organisms as Streptococccus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pyrogenes, Corynebacterium diphtheriae, Listeria monocytogenes, Bacillus anthracis, Clostridium tetani, Clostridium botulinum, Clostridium perfringens, Neisseria meningitidis, Neisseria gonorrhoeae, Streptococcus mutans, Pseudomonas aeruginosa, Salmonella typhi, Haemophilus parainfluenzae, Bordetella pertussis, Francisella tularensis, Yersinia pestis, Vibrio cholerae, Legionella pneumophila, Mycobacterium tuberculosis, Mycobacterium leprae, Treponema pallidum, Leptospirosis interrogans, Borrelia burgdorferi, Camphylobacter jejuni, and the like; antigens of such viruses as smallpox, influenza A and B, respiratory syncytial virus, parainfluenza, measles, HIV, varicella-zoster, herpes simplex 1 and 2, cytomegalovirus, Epstein-Barr virus, rotavirus, rhinovirus, adenovirus, papillomavirus, poliovirus, mumps, rabies, rubella, coxsackieviruses, equine encephalitis, Japanese encephalitis, yellow fever, Rift Valley fever, hepatitis A, B, C, D, and E virus, and the like; antigens of fungal, protozoan, and parasitic organisms such as Cryptococcus neoformans, Histoplasma capsulatum, Candida albicans, Candida tropicalis, Nocardia asteroides, Rickettsia ricketsii, Rickettsia typhi, Mycoplasma pneumoniae, Chlamydial psittaci, Chlamydial trachomatis, Plasmodium falciparum, Trypanosoma brucei, Entamoeba histolytica, Toxoplasma gondii, Trichomonas vaginalis, Schistosoma mansoni, and the like. These antigens may be in the form of whole killed organisms, peptides, proteins, glycoproteins, carbohydrates, or combinations thereof.

While practically any bioactive agent, small molecule, or drug may benefit from the teachings herein, certain pharmaceutical compositions will find particular utility in the inventive compositions. Proteins such as insulin that are not generally stable in the gastrointestinal system may be encapsulated using the techniques of the invention. For example, diabetics could swallow a capsule containing microparticles or nanoparticles having encapsulated insulin. The particles would adhere to the mucosa and pass through the mucosal layer into the blood stream, where they would gradually release insulin. Peptides and small molecules may be delivered in the same manner. Other biomolecules involved in metabolic disorders may also be delivered using the techniques of the invention. For example, phenylalanine hydroxylase and/or

tyrosine may be administered to phenylketonurics. Nutritional and enzymatic supplements may be provided to patients with maple syrup urine disease. The techniques of the invention may be exploited to provided enzyme replacement therapy to treat a host of metabolic diseases including but not limited to Gaucher disease, Fabry disease, Niemann-Pick disease, cystic fibrosis, mucopolysaccharidosis, Tay-Sachs disease, Hurler syndrome, many forms of muscular dystrophy, including Pompe disease, and lysosomal storage disorders (see, for example, Sly, "Enzyme replacement therapy for lysosomal storage disorders: successful transition from concept to clinical practice," *Mo Med.* 2004 Mar-Apr;101(2):100-4; Desnick, et al., "Enzyme replacement and enhancement therapies: lessons from lysosomal disorders," *Nat Rev Genet.* 2003 Feb;4(2):157).

For patients who take a drug every day, the compositions of the invention can reduce the frequency with which patients have to take the drug. For example, a patient could take a pill once a week or once a month instead of daily. In one embodiment, controlled release particles produced using the invention may be used to deliver contraceptive drugs to patients. Instead of taking a pill every day, the formulations of the invention may be used to provide a weekly or monthly dose regimen. Estrogen replacement therapy may be administered in the same manner. For example, female reproductive hormones, for example, estrogen and progesterone, may be formulated as particles using the techniques of the invention.

In one embodiment of the present invention, the agent to be delivered may be a mixture of agents. For example, an antibiotic may be combined with an inhibitor of the enzyme commonly produced by bacteria to inactivate the antibiotic (e.g., penicillin and clavulanic acid). In one embodiment, different active agents may be compounded into particles, and then mixtures of different particles may be combined with a delivery vehicle in specific ratios using the techniques described below to provide different combinations of active agents to patients. For example, cyclic contraceptives work by providing a different ratio of reproductive hormones to patients over the course of three weeks, simulating the manner in which the ratio of estrogen and other hormones vary over the course of a menstrual cycle. Rather than preparing particles with different ratios of estrogen and progesterone, different ratios of particles encapsulating estrogen and progesterone may be compounded into single dosage units.

The techniques of the invention provide improved bioavailability to the compounds delivered thereby. The bioavailability of the active agent may be determined using standard pharmacokinetic techniques known to those skilled in the art. For example, the concentration of the active agent in the bloodstream or of the agent or its derivatives in urine may be measured periodically and used to calculate AUC (area under the curve).

The pharmaceutically active agent may be coordinated with or encapsulated in a polymer, for example, a biodegradable, biocompatible polymer. A variety of biodegradable polymers are well known to those skilled in the art. Exemplary synthetic polymers suitable for use with the invention include but are not limited to poly(arylates), poly(anhydrides), poly(hydroxy acids), polyesters, poly(ortho esters), polycarbonates, poly(propylene fumerates), poly(caprolactones), polyamides, polyphosphazenes, polyamino acids, polyethers, polyacetals, polylactides, polyhydroxyalkanoates, polyglycolides, polyketals, polyesteramides, poly(dioxanones), polyhydroxybutyrates, polyhydroxyvalyrates, polycarbonates, polyorthocarbonates, poly(vinyl pyrrolidone), biodegradable polycyanoacrylates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), poly(methyl vinyl ether), poly(ethylene imine), poly(acrylic acid), poly(maleic anhydride), biodegradable polyurethanes and polysaccharides. U.S. Patents that describe the use of polyanhydrides for controlled delivery of substances include U.S. Pat. No. 4,857,311 to Domb and Langer, U.S. Pat. No. 4,888,176 to Langer, et al., and U.S. Pat. No. 4,789,724 to Domb and Langer.

Naturally-occurring polymers, such as polysaccharides and proteins, may also be employed. Exemplary polysaccharides include alginate, starches, dextrans, celluloses, chitin, chitosan, hyaluronic acid and its derivatives; exemplary proteins include collagen, albumin, and gelatin. Polysaccharides such as starches, dextrans, and celluloses may be unmodified or may be modified physically or chemically to affect one or more of their properties such as their characteristics in the hydrated state, their solubility, or their half-life in vivo.

In other embodiments, the polymer includes polyhydroxy acids such as polylactic acid (PLA), polyglycolic acid (PGA), their copolymers poly(lactic-co-glycolic acid) (PLGA) in any ratio, for example, 50:50 poly(DL-lactic-co-glycolic acid), and mixtures of any of these. These polymers are among the synthetic

polymers approved for human clinical use as surgical suture materials and in controlled release devices. They are degraded by hydrolysis to products that can be metabolized and excreted. Furthermore, copolymerization of PLA and PGA offers the advantage of a large spectrum of degradation rates from a few days to several years by simply varying the copolymer ratio of glycolic acid to lactic acid, which is more hydrophobic and less crystalline than PGA and degrades at a slower rate. The chirality of the two polymers may also be manipulated.

Co-polymers, mixtures, and adducts of any of the polymers may also be employed. For example, block co-polymers may have regions that are chosen to optimize their association with the magnetic particle or the pharmaceutically active agent. Alternatively or in addition, polymers may be chemically modified to have particular functional groups. For example, polymers may be functionalized with hydroxyl, amine, carboxy, maleimide, thiol, N-hydroxy-succinimide (NHS) esters, azide groups, or other charged or chargeable chemical groups. These groups may be used, for example, to impart a particular charge or other chemical property to the polymer, to facilitate association of the polymer with the pharmaceutically active agent and/or the magnetic particle, or to modify the polymer's response to the physiological environment. Alternatively or in addition, poly(ethylene glycol) or poly(propylene glycol) groups may be attached to the polymer or to the particle surface.

One skilled in the art will recognize that the molecular weight and the degree of cross-linking may be adjusted to control the decomposition rate of the polymer. Methods of controlling molecular weight and cross-linking to adjust release rates are well known to those skilled in the art.

A variety of methods of making particles in which active agents are encapsulated are well known to those skilled in the art. For example, a double emulsion technique may be used to combine a polymer and a pharmaceutically active agent in particles. The magnetic particle may be co-encapsulated with the pharmaceutically active agent in a polymer particle. Alternatively, particles may be prepared by spray-drying. The polymer particles may be between 1 micron and 1 mm in diameter or even larger. For example, the polymer particles may be between 1 and 10 micron, between 10 and 100 micron, between 100 and 500 micron, or between 500 micron and 1 mm in diameter.

The pharmaceutically active agent may also be covalently or non-covalently associated with the polymer in a conjugate without actually encapsulating the agent with the polymer. For example, PLGA may be modified with a carboxylate group and coupled with an aminated pharmaceutically active agent using a coupling reagent such as EDC or DCC. Alternatively, PLGA may be modified to have an activated NHS ester which can then be reacted with an amine group on the pharmaceutically active agent. Either the polymer or the agent may be modified to include reactive groups such as hydroxyl, amine, carboxyl, maleimide, thiol, NHS ester, azide, or alkyne. Standard coupling reactions may then be used to couple the modified material to a second material having a complementary group (e.g., a carboxyl modified core coupled to an aminated coating material). In another example, a charged pharmaceutically active agent may be associated with a polymer carrying the opposite charge using electrostatic interactions. The polymer may be chemically modified to provide the appropriate charge. In addition to electrostatic interactions, other non-covalent interactions may also be used to immobilize a coating. Additional non-covalent interactions include but are not limited to the following:

1) Affinity Interactions: For example, biotin may be attached to the polymer and streptavidin may be attached to the pharmaceutically active agent; or conversely, biotin may be attached to the agent and the streptavidin may be attached to the surface of the polymer. The biotin group and streptavidin may be attached to the polymer or to the agent via a linker, such as an alkylene linker or a polyether linker. Biotin and streptavidin bind via affinity interactions, thereby maintaining the association between the polymer and the agent.

2) Metal Coordination: For example, a polyhistidine may be attached to the pharmaceutically active agent, and a nitrilotriacetic acid can be attached to the polymer. A metal, such as Ni^{+2}, will chelate the polyhistidine and the nitrilotriacetic acid, thereby maintaining the association between the polymer and the agent.

3) Physical Adsorption: For example, a hydrophobic tail, such as polymethacrylate or an alkyl group having at least about 10 carbons, may be attached to the pharmaceutically active agent. The hydrophobic tail will adsorb onto the surface of a hydrophobic polymer, such as a polyorthoester, polysebacic anhydride, unmodified poly(lactic acid),or polycaprolactone, thereby maintaining the association between the polymer and the agent.

4) Host-Guest Interactions: For example, a macrocyclic host, such as cucurbituril or cyclodextrin, may be attached to the polymer, and a guest group, such as an alkyl group, a polyethylene glycol, or a diaminoalkyl group, may be attached to the pharmaceutically active agent, or vice versa. In one embodiment, the host and/or the guest molecule may be attached to the agent or the polymer via a linker, such as an alkylene linker or a polyether linker.

5) Hydrogen Bonding Interactions: For example, an oligonucleotide having a particular sequence may be attached to the surface of the polymer, and an essentially complementary sequence may be attached to the pharmaceutically active agent. Alternatively, where the pharmaceutically active agent includes a nucleic acid, an oligonucleotide having an essentially complementary sequence may be attached to the polymer. The agent will then bind to the polymer via complementary base pairing with the oligonucleotide attached to the polymer. Two oligonucleotides are essentially complimentary if about 80% of the nucleic acid bases on one oligonucleotide form hydrogen bonds via an oligonucleotide base pairing system, such as Watson-Crick base pairing, reverse Watson-Crick base pairing, Hoogsten base pairing, etc., with a base on the second oligonucleotide. In some embodiments, it is desirable for an oligonucleotide sequence attached to the controlled release polymer system to form at least about 6 complementary base pairs with a complementary oligonucleotide attached to the nucleic acid ligand. For example, a poly(cytosine) tag may be attached to the polymer and a poly(guanine) tag may be attached to the pharmaceutically active agent. Where the polymer is a sugar, hydroxyl groups on sugars such as glucose and galactose will hydrogen bond with polar moieties on pharmaceutically active agents. Sugar dimers or oligomers may be used as well.

The polymer-agent conjugate or polymer particle (which encapsulates a pharmaceutically active agent), may be associated with the magnetic particle in the same ways in which the polymer is associated with the pharmaceutically active agent. For example, the magnetic particles may be co-encapsulated with the agent. Alternatively or in addition, the magnetic particles may be covalently or non-covalently associated with the polymer in the same manner as the pharmaceutically active agent is associated with the polymer. Magnetite particles functionalized with biotin, streptavadin, Protein A, Protein G, poly(adenine), and various antibodies are available from Polysciences. Particles functionalized with amine and carboxyl are

also available and may be functionalized with any of the non-covalent coupling groups described above or used as is. Magnetic particles may be functionalized using silanes to retain an appropriate group on their surface. For example, amine, carboxyl, or other moieties that participate in coupling reactions may be retained on the surface of a magnetic particle using an appropriately terminated organosilane.

Alternatively or in addition, the pharmaceutically active agent may be directly associated with the magnetic particle. Again, the same mechanisms discussed above to associate the agent with a polymer may be used to associate the agent with a magnetic particle. In one embodiment, an agent carrying a negative charge is associated with an amine-functionalized particle by electrostatic interactions. In another embodiment, a biotinylated agent is associated with a streptavadin-functionalized particle, or vice versa.

The magnetic particle may be fabricated from any material that will be retained in the intestines in response to an externally applied magnetic field. The magnitude of the field may be varied depending on the application. For example, where a pharmaceutically active agent is repeatedly administered, a smaller field, such as that available from a wearable magnet, such as a belt buckle or a belt or other garment having magnets attached to it, may be more desirable. In such applications, it may be desirable that the magnetic particle exhibit a strong response to a magnetic field. In other applications, it may be desirable to apply a stronger magnetic field, such as that available from an MRI apparatus. In such cases, highly magnetic or magnetically susceptible particles may not be desirable. Any permanently magnetic material may be employed as a magnet to retain the magnetic particles in situ. Alternatively or in addition, an electromagnet may be employed to generate a magnetic field at desired times. The magnetic field may be as low as one Gauss to tens, hundreds, or thousands of Gauss. In some embodiments the magnetic field is one or two Tesla or greater.

The magnetism of the magnetic particle may be due to any magnetic phenomenon, for example, paramagnetism, superparamagnetism, ferromagnetism, or ferrimagnetism. The magnetic particle may be metallic or ceramic. Exemplary metallic materials include but are not limited to iron, nickel, cobalt, and magnetic alloys of these metals with each other and with such materials as silicon, copper, chromium, molybdenum, boron, neodymium, samarium, etc., e.g., neodymium-iron-

boron, samarium-cobalt, 3 and 4% Si-Fe alloys, and nickel iron alloys. Exemplary ceramic materials include ferrites, garnets, magnetoplumbites ($MO{\cdot}6Fe_2O_3$, where M is a divalent metal), and other ceramics, including but not limited to magnesium ferrite, magnesium zinc ferrite, manganese ferrite, manganese iron ferrite, manganese-zinc ferrite, nickel ferrite, lithium ferrite, magnetite, yttrium iron garnet, aluminum substituted YIG, chromium substituted YIG, lanthanum iron garnet, praseodymium iron garnet, and garnet mixtures.

The magnetic particle may be any size sufficiently small to be combined with a pharmaceutically acceptable carrier. For example, the particle may be between 5 nm and 5 mm in diameter, for example, between 5 and 100 nm, between 100 and 500 nm, between 500nm and 1 micron, between 1 micron and 10 micron, between 10 micron and 100 micron, between 100 micron and 500 micron, between 500 micron and 1 mm, or between 1 mm and 5mm. Smaller particles may be able to penetrate through the intestinal wall into the bloodstream. Larger particles can remain resident in the intestines for a desired amount of time and be eliminated after they have finished discharging their associated pharmaceutically active agent.

Larger magnetic particles, for example, about 5 μm or greater, exhibit increased magnetic responsiveness and substantial surface area. Such particles are well-suited to be coordinated with one or more polymer particles by charge interactions. For example, PLGA-insulin particles exhibit a negative surface charge. Magnetic particles functionalized with charged amine groups exhibit strong interactions with the PLGA-insulin particles. The magnetic particles are irregularly shaped and can interact with several polymer particles. One skilled in the art will recognize that the amount of polymer particles a magnetic particle can interact with will depend on the relative surface areas of the two particles. Magnetic particles may be purchased commercially with a particular surface charge or may be functionalized with charged chemical groups using any of the techniques described herein or otherwise known to those of skill in the art.

The magnetic particles may be fabricated using any method known to those skilled in the art. Exemplary methods of fabricating magnetic particles are disclosed in U.S. Patent No. 5,071,076, Hyeon, *Nature Materials 3, 891–895 (2004)*, U.S. Patent No. 4,672,040, and U.S. Patent No. 3,933,997, the contents of all of which are incorporated herein by reference. Magnetic particles may be fabricated with organic

coatings, as described in U.S. Patent No. 4,770,183, the contents of which are incorporated herein by reference.

Once the magnetic particles have been associated with the desired pharmaceutically active agent, they may be combined with pharmaceutically acceptable carriers to form a pharmaceutical composition. While the composition may be injectable or administrable as a suppository, it is preferable that the composition be orally administrable, either through ingestion or as an inhalant. One skilled in the art will recognize that the optimum particle size may vary depending on the nature of the drug being delivered. The studies described below may be used to determine the optimal particle size.

As used herein, the term "pharmaceutically acceptable carrier" means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. *Remington's Pharmaceutical Sciences* Ed. by Gennaro, Mack Publishing, Easton, PA, 1995, discloses various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, sugars such as lactose, glucose, and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; detergents such as TWEEN™ 80; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen- free water; isotonic saline; Ringer's solution; ethyl alcohol; and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate. Coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and/or antioxidants can also be present in the composition, according to the judgment of the formulator.

The pharmaceutical compositions of the invention can be administered to a patient by any means known in the art including oral and parenteral routes. The term "patient", as used herein, refers to humans as well as non-humans, including, for example, mammals, birds, reptiles, amphibians, and fish. Preferably, the non-humans

are mammals (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a primate, or a pig). Non-edible compositions may be administered by injection (e.g., intravenous, subcutaneous or intramuscular, intraperitoneal injection), rectally, vaginally, topically (as by powders, creams, ointments, or drops), or by inhalation (as by sprays).

Powders and sprays can contain, in addition to the magnetic particles, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates, and polyamide powder, or mixtures thereof. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons.

Pharmaceutical compositions for oral administration can be liquid or solid. Liquid dosage forms suitable for oral administration of inventive particles include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to an encapsulated or unencapsulated particle, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants, wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents. As used herein, the term "adjuvant" refers to any compound which is a nonspecific modulator of the immune response. In certain preferred embodiments, the adjuvant stimulates the immune response. Any adjuvant may be used in accordance with the present invention. A large number of adjuvant compounds is known in the art (Allison *Dev. Biol. Stand.* 92:3-11, 1998; Unkeless et al. *Annu. Rev. Immunol.* 6:251-281, 1998; and Phillips et al. *Vaccine* 10:151-158,1992).

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the encapsulated or unencapsulated particle is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or (a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, (b)

binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, (c) humectants such as glycerol, (d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, (e) solution retarding agents such as paraffin, (f) absorption accelerators such as quaternary ammonium compounds, (g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, (h) absorbents such as kaolin and bentonite clay, and (i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets, and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art.

It will be appreciated that the exact dosage of the inventive particle is chosen by the individual physician in view of the patient to be treated. In general, dosage and administration are adjusted to provide an effective amount of the desired active agent to the patient being treated. As used herein, the "effective amount" of a substance refers to the amount necessary to elicit the desired biological response. As will be appreciated by those of ordinary skill in the art, the effective amount of encapsulated active agent may vary depending on such factors as the desired biological endpoint, the active agent to be delivered, the target tissue, the route of administration, etc. For example, the effective amount of inventive particles containing an anti-cancer drug might be the amount that results in a reduction in tumor size by a desired amount over a desired period of time. Additional factors which may be taken into account include the severity of the disease state; age, weight and gender of the patient being treated; diet, time and frequency of administration; drug combinations; reaction sensitivities; and tolerance/response to therapy.

The magnetic particles may be compounded with a carrier in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of conjugate appropriate for

the patient to be treated. It will be understood, however, that the total daily usage of the compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. For any particle composition, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. Therapeutic efficacy and toxicity of particle materials and the drugs delivered thereby can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED_{50} (the dose is therapeutically effective in 50% of the population) and LD_{50} (the dose is lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use.

Examples

Example 1: General

Human insulin (Humulin R, 500 U/mL), a model drug used in this study, was purchased from drugstore.com. Poly(DL-lactide-co-glycolide) (50/50) with acid terminal groups (PLGA, inherent viscosity 0.18 dl/g) was obtained from Absorbable Polymers International (Pelham, AL). Poly(vinyl alcohol) (PVA, MW 30 kDa-70 kDa), Iron (III) chloride and Iron (II) chloride were purchased from Sigma/Aldrich chemical company and used as received. FITC-magnetic polystyrene beads (fluorescent YG superparamagnetic microspheres, 1-2 μm) were purchased from Polysciences, Inc. Scanning electron microscopy (SEM) was recorded on a JEOL JSM 6060 system. Fluorescent images were taken on an Axiovert 200 inverted microscope (Zeiss). Magnets (Neodymium iron boron rare earth magnets, 1" × 1" × 0.5"(thickness), Grade N40, magnetized through the thickness) were purchased from Amazing Magnets (amazingmagnets.com). (3-[^{125}I]iodotyrosyl^{A14}) insulin (human recombinant) was obtained from Amersham Biosciences (Piscataway, NJ). Radioactivities of ^{125}I-insulin or MPs were analyzed on a TRI-CARB Liquid Scintillation Analyzer (Model 2200CA, Packard Instrument Company, Downers

Grove, IL). Hionic-Fluor cocktail and Scinti-Safe scintillation cocktails and Solvable tissue solubilizer were purchased from Packard Instrument Company. Magnetite nanocrystals (12 nm) were synthesized following the published procedure (Mehta, et al., *Biotechnol. Tech.*, 1997, **11**:493-496, the contents of which are incorporated herein by reference). Briefly, 27g of $FeSO_4 \cdot 7H_2O$ in 100 mL doubly-distilled water and 57 g $FeCl_3 \cdot 6H_2O$ in 100 mL water were thoroughly mixed and added to 8M ammonium hydroxide at room temperature under continuous stirring. The pH was maintained at approximately 10 by adding ammonium hydroxide during the reaction. The resulting black particles exhibited a strong magnetic response. Impuries such as chloride and sulfate ions were removed by washing with copious amounts of hot distilled water. BALB/C mice were purchased from Charles River laboratory (Wilmington, MA).

Example 2: Preparation of PLGA microparticles (MPs) encapsulating Humulin R or ^{125}I-Insulin

Insulin and magnetite co-encapsulated MPs were prepared using the water-in-oil-in-water solvent evaporation procedure (double emulsion). 50 μL of the Humulin R solution (500 U/mL) was emulsified with 50 mg PLGA in dichloromethane (1 mL) and 1-5 mg (e.g., 2-10 wt%) of magnetite nanocrystals (10-15 nm in diameter as measured by transmission electron microscopy, not shown) for 30s using a probe sonicator at 10W. The first emulsion was transferred to a 50 mL aqueous PVA solution (1 % w/v) and homogenized at 8000 rpm for 1 minute. The resulting emulsion was immediately poured into a 150 mL aqueous PVA solution (0.3 % w/v) with gentle stirring. Organic solvent was removed through slow evaporation at room temperature for 2.5 h. The resulting insulin and magnetite co-encapsulated MPs were isolated as a gray to light-brown solid by centrifugation at 3200 rpm and at 10 °C for 10 minutes, washed twice with double-distilled water and lyophilized. The yields of MPs are in a range of 50-60% with encapsulation efficiency 60-80%. Loading of insulin was determined by protein BCA Assay (PIERCE) by dissolving MPs using a mixture of acetonitrile and water.

Preparation of all other MPs (either PLGA or poly(lactic acid) (PLA)) with encapsulated insulin (either Humulin R or ^{125}I-inuslin) either with or without magnetite are the same as above using appropriate materials

Example 3: Characterization of insulin-containing PLGA MPs

The sizes of MPs were measured on a Beckman Coulter Multisizer^{TM}-3. Electrophoretic mobilities were measured at 25 °C on a ZetaPALS dynamic light scattering system (Brookhaven Instruments Corporation) using BIC PALS zeta potential analysis software. Zeta potentials were calculated using the Smoluchowsky model.

Encapsulation efficiency was determined using MPs encapsulating ^{125}I-labeled insulin. The supernatant after centrifugation was collected and measured along with an aliquot of MPs by liquid scintillation counting. The encapsulation efficiency was calculated by the difference between the total amount of radioactivity in the initial solution and the remaining amount in the supernatant.

Example 4: Oral administration of FITC-paramagnetic beads

Mice were fasted for 12 h and then gavaged a solution of fluorescent YG superparamagnetic microspheres (5 mg/ 200 μL PBS). Forty minutes after administration, mice were restrained and a magnet was applied to their abdominal area. Control mice were restrained in the same way but no magnet was applied. Mice were sacrificed 6h after administration. Small intestines of the mice from both groups were collected, dissolved in Solvable and analyzed by fluorescent microscopy.

The intestinal transit of particulate drug delivery vehicles is relatively fast in mice. The majority of the particles travel through the intestine in about 2 hours[14]. This rapid transit time prevents delivery vehicles from being absorbed onto the surface of intestinal epithelium. In order to slow down the transit of polymer microparticles, we have developed magnetically responsive polymer particles by incorporating magnetites. The magnetically responsive drug carriers are localized in the intestine by the application of an external magnetic field, resulting in either enhanced absorption of drug carriers or absorption of the locally released protein drugs.

To evaluate whether the external magnet can retain magnetic particles in the intestine, we used fluorescent, paramagnetic beads as a model drug delivery system. The retention of MPs in the intestines is significantly improved in the magnet-applied

mice compared to the control mice. The presence of fluorescent particles in the small intestines was visualized using fluorescent microscopy (Figure 2). Mice that are applied an external magnet field showed much stronger fluorescent activity than mice that are not applied an external magnetic field (Figure 2B and 2C).

Example 5: Oral administration of ^{125}I-Insulin-encapsulated PLGA MPs and Humulin R-PLGA-magnetite (2 wt%)-PLGA MPs

A first group of mice were fasted for 12 h and then orally administered with 1 μCi ^{125}I-insulin-magnetite (2 wt%)-PLGA microparticles in 200 μL water. Forty minutes after administration, mice were restrained in the presence or absence of magnetic field. A magnet was placed near the abdominal area with magnetization surface facing abdomen. Mice were sacrificed at 6 and 12 hours. The small intestine was homogenized, and approximately 100mg of the homogenized intestine mixture was placed in a 20 ml scintillation vial. Solvable (2 ml) was added to the vial and then incubated until the tissue completely dissolved (6-10 h) at 55 °C. After the solution was cooled to room temperature, an EDTA-disodium solution (0.05 ml, 0.1M) was added to the vial, followed by 0.2 ml 30% hydrogen peroxide by slow addition. The solution was agitated gently between additions of hydrogen peroxide to allow reaction and foaming to subside. The solution was then incubated in the oven at 55 °C for another hour to result in a colorless solution. Scintillation cocktail (10 ml Hionic-Fluor) was added to the liquid. Samples were acclimated to light and temperature conditions in the counter for 30 minutes prior to counting, and the radioactivity was measured on a TRI-CARB Liquid Scintillation Analyzer

A second group of Balb/c mice were fasted for 12 hours and then orally administered with Humulin R-PLGA-magnetite (8 wt%)-PLGA MPs at 100 U/kg. Five mice were assigned to each group such that the mean values of their initial glucose levels were identical. Humulin R-PLGA-magnetite (100 U/kg) in 400 μl water was administrated orally using syringe with gavage needles. Control mice were administered with 200 μl water only. 90 minutes after administration, mice were restrained in the presence or absence of a magnetic field (similar as above). The glucose level of each mouse was monitored over time by collecting blood from the tail vein and measuring using the One Touch Ultra glucose monitor (Lifescan, Milpitas, California). As an intravenous standard, 0.5 U/kg of Humulin R was

injected into the tail vein, and the glucose level was monitored as described above. To calculate the biological effect, the area under curve (AUC) for the plot of decrease in blood glucose levels (%) over time (h) were calculated using the trapezoidal method. Bioavailability (f) was calculated based on equation (1) (7,15,16).

$$f = \frac{\frac{AUC_{Oral} \times weight_{oral}}{Dose_{oral}}}{\frac{AUC_{i.v.} \times weight_{i.v.}}{Dose_{i.v.}}} \times 100\% \qquad (1)$$

To measure *in vivo* insulin concentration, the above experiment was performed, blood (~25 μL) samples were collected into Startedt serum gel microtubes. Serum (5 μL) was analyzed for insulin content by the Mercodia insulin ELISA kit (ALPCO Diagnostics, Inc., Windham, NH). As an intravenous standard, 2 U/kg of Humulin R was injected into the tail vein, and insulin concentration was similarly detected. Bioavailability (f) was also calculated based on equation (1) as described above.

Results in the text are expressed as mean ± S.D. unless otherwise stated. Data was analyzed using ANOVA. For statistical tests, a *P*-value of 0.05 or less was considered significant.

A third group of mice were fasted for 12 h and then orally administered with Humulin R-PLGA-magnetite (2 wt%)-PLGA MPs at 50 unit/kg. Glucose levels were measured using Ascensia Breeze Blood Glucose Monitoring System (Bayer). Four mice were assigned to each group such that the mean values of their initial glucose levels were consistent. Humulin R-PLGA-magnetite (50 unit/kg) in 200 μL PBS were administrated orally using gavage needles. Control mice were administered with 200 μL PBS only. The glucose level of each mouse was monitored over time.

Example 6: Interaction between BioMag-NH_2 and Dextran-Rhodamine-PLA-COOH MP

We studied the interaction between the BioMag-NH_2 particles (6 μm average diamerter magnetites with positive surface charge purchased from Polysciences, Inc.) and Dextran-Rhodamine-PLA-COOH MPs (negative surface charge). When 5 mg BioMag-NH_2 and 2.5 mg Dextran-Rhodamine-PLA-COOH MP were mixed, we observed immediate immobilization of PLA MPs by the BioMag particles. The

particle complexes were washed with 6 × 1 mL DI water to remove unbound PLA-MPs. Washing solution was removed by pipettor after immobilization of BioMag-NH_2/PLA MPs complexes with a magnet. The complexes were analyzed under fluorescence microscope (Figure 1). The negatively charged of Dextran-Rhodamine-PLA-COOH MP and positively charged BioMag-NH_2 form aggregates likely through charge interaction (Figure 4C).

Example 7: Interaction between BioMag (1 μm, Polysciences, Inc.) and PLA-COOH MP

We also studied the interaction between PLA MPs and BioMag with smaller size (1 μm). The polymer and BioMag particles we used are:

Negatively charged MP:	PLA-COOH MP (zeta potential -32.38 ± 1.2, Figure 6A)
Positively charged MP:	PLA-COOH MP modified with 0.5% Chitosan (zeta-potential +52.94 ± 0.78)
Positively charged BioMag:	BioMag- NH_2 (Figure 6B)
Negatively charged BioMag:	BioMag-COOH (Figure 6C)

Because PLA MP and BioMag have different sizes, they are easily differentiated under SEM (Figure 6A-C). Polymer MPs have regular spherical shape, while both BioMag-NH_2 and BioMag-COOH have irregular shapes. When the polymer MPs were mixed with oppositely charged BioMag, they form very stable complexes through charge interaction as shown in Figure 6D (negatively charged BioMag-COOH with positively charged PLA-Chitosan MP) and Figure 6E (positively charged BioMag-NH_2 with negatively charged PLA-COOH MP). The spherical surfaces of polymer MPs are coated with BioMag particles with opposite charge and thus become less smooth.

Mixing BioMag-COOH and PLA-COOH MP, both are negatively charged, did not form complexes as observed in Figure 3D and 3E. The majority of PLA-COOH MPs were removed during washing step; therefore the materials observed under SEM are mainly the BioMag-COOH (Figure 6F). Some remaining PLA-COOH MPs observed have very smooth surfaces (Figure 6F), indicating that there is

no interaction between the negatively charged PLA-COOH MP and the negatively charged BioMag-COOH.

Example 8: In vitro Magnetic Responsiveness

The retention of microparticles in the small intestine was modeled *in vitro* in flow conditions. An apparatus approximating the physiology of the mouse small intestine was constructed (Figure 7).

The length (330 mm) and diameter (2 mm) of the tubing were selected to simulate *in vivo* conditions for mice (17). ^{125}I-Insulin-PLGA-COOH (1.25 mg) and BioMag-NH_2 (1.25 mg) complex were injected into the system. PBS was flowed through the system at approximately 0.8 mL/min, to test retention of particles in greater than physiological flow conditions (typically 30 μL/min). One tube was placed in the proximity of a magnetic, and one tube served as the control. The eluted liquid was collected at 5 minute intervals (about 4 mL). Scintillation cocktail (10 mL ScintiSafe) was added to the liquid, and the radioactivity was measured in a liquid scintillation counter. The cumulative percentage of particles eluted (based on 100% injected dose) is shown on Figure 8.

Example 9: Histology analysis

BioMag-NH_2 (1 μm, 5 mg) and BioMag-COOH (5 mg) were mixed with rhodamine-dextran encapsulated PLA-COOH MPs (10 mg, zeta potential = -32) and Chitosan-modified, rhodamine-dextran encapsulated PLA-COOH MPs (zeta potential = +53), respectively. Condensation of MP with BioMag-NH_2 completed immediately after mixing since the PLA particles disappeared from solution after applying a magnetic field at the bottom of the mixing eppendorf tube. A 200 μL water solution of 7.5 mg complexes was gavaged into mice. One group of mice was restrained 40 minutes after administration and a magnetic field was applied to the abdominal area of each mouse. The other group was restrained in the absence of external magnets. Mice were euthanized after 8 h. The small intestines were collected for analysis. The difference between magnet applied and non-applied groups is very obvious as evidenced by leaking of brown magnetic materials from the intestine during fixation (Figure 9).

The small intestine was harvested from each animal and washed in PBS. The tissue was fixed for one hour in 10% formalin and washed in 30% Sucrose before being frozen in OCT media (Sakura Finetek). Cryosections (5-micron thickness) were cut of the tissue, and images were taken at 20X under halogen and fluorescent settings (rhodamine filtered). For each tissue, an overlay image was produced showing rhodamine-encapsulating microspheres in the small intestine wall.

Both complexes (BioMag-COOH/chitosan-coated rhodamine-dextran-PLA-COOH MP and BioMag-NH_2/rhodamine-dextran-PLA-COOH MP) showed virtually no retention of fluorescent particles after eight hours in the absence of magnets (Figure 10A and 10B). This result is consistent with the normal time of residence of material in the small intestine. In the groups in which the magnets were applied, both complexes showed significant retention (see Figure 10C-F).

Example 10: In vivo distribution of BioMag-NH_2-^{125}I-Insulin-PLA-COOH MP complexes

Radioactivity in the small intestine of magnet-applied mice is 1460% times higher than that of magnet-not-applied mice. The total radioactivity detected in blood (assuming 2 mL total blood volume in each mouse) in magnet-applied mice is 90% higher than in magnet-not-applied mice (Figure 11).

Example 11: Efficacy study

We formulated insulin-containing-PLGA-COOH MPs and NPs with BioMag-NH_2 (6 μm) and tested these particle complexes in vivo for oral insulin delivery. Forty minutes after the administration of these particle complexes, a magnetic field was created externally to the mouse abdomen in the intestine area to retain or slow down the transit of these particle complexes.

A single administration of 50 U/kg of insulin-PLGA microparticle/BioMag-NH_2 to fasted mice resulted in a 60% reduction of glucose level from its original level at 4h in the presence of external magnetic field (Figure 12). The glucose level remained at this level for at least another16 hours. As a comparison, mice administered in the same way in the absence of an external magnet show 45% reduction of their whole blood glucose concentration at 4 h (Figure 12). Both groups

show better efficacy than MP group alone, which resulting in 25% glucose reduction at the same dose in a separate study (data not shown)

A single administration of 50 U/kg of insulin-PLGA nanoparticle/BioMag-NH_2 to fasted mice resulted in a 45% reduction of glucose level at 4h in the presence of external magnetic field (Figure 12). The glucose level gradually decreased to a level that is 60% reduction from its original level at 20 h. As a comparison, mice administered in the same way in the absence of an external magnet show 28% reduction of their whole blood glucose concentration at 4 h and reach the minimum reduction (29%) at 10 h (Figure 12).

Bioavailibilities were calculated based on the AUC of glucose reduction for treatment groups and AUC of glucose reduction of intravenous administration using insulin alone. The bioavailabilities are 5.4 ± 1.79% and 4.07 ± 1.19% for the BioMag-NH_2/Humulin R-PLGA-COOH MPs in the presence and absence of external magnetic field, respectively. It is surprising that even the BioMag-NH_2/MP with no-magnetic field gave very high bioavailability, probably because of overdosing. The bioavailabilities are 4.21 ± 2.13% and 1.16 ± 0.69% for the BioMag-NH_2/insulin-PLGA-COOH NPs in the presence and absence of external magnetic field, respectively.

Example 12: In Vivo Retention Study of PolyMP/MagMP Complex

MagMP+ (5 mg) were mixed with rhodamine-dextran encapsulated PolyMP (10 mg, zeta potential = -32). A 200 μL water solution of 7.5 mg of MagMP-PolyMP complexes was gavaged to mice. One group of mice was restrained 40 minutes after administration and a magnetic field was applied to the abdominal area of each mouse. The other group was restrained in the absence of external magnetic field. Mice were euthanized after 8 h. The small intestine was harvested from each animal and washed in PBS. The tissue was fixed for one hour in 10% formalin and washed in 30% Sucrose before being frozen in OCT media (Sakura Finetek). Cryosections (5-micron thickness) were cut of the tissue, and images were taken at 20X under halogen and fluorescent settings (rhodamine filtered). For each tissue, an overlay image was produced showing rhodamine-encapsulated PolyMP in the small intestine wall. For quantitative analysis, mice were similarly gavaged with ^{125}I-Insulin encapsulated PolyMP. After 19 h, mice were euthanized and blood was drawn by cardiac puncture.

Intestines were harvested and solubilized. Blood and tissue was de-colored and analyzed for ^{125}I content by liquid scintillation counting (Packard Tri-Carb LSC).

Example 13: Acute Toxicity Histological Analysis

Mice were gavaged with magnetite-PLGA MPs and restrained in the presence of the magnetic field, as described in the above efficacy study. After 24 hours, mice were sacrificed, and tissues were harvested for analysis of acute toxicity. For comparison, mice not administered with NPs were similarly sacrificed and treated. Tissue sections from the small intestine, spleen, kidney, and liver were fixed in 10% formalin and processed for histology as per standard techniques. Sections were stained with Hematoxylin and Eosin (H&E) and investigated for acute inflammation and particle toxicity. Sections were stained for colloidal iron using the Mallory Method with Prussian Blue to determine magnetite uptake by the body. Magnetite-PLGA MPs were separately embedded and stained as a positive control for the iron staining.

Example 14: Bioavailability Studies

We formulated insulin-containing PolyMP- with MagMP+ and tested these particle complexes *in vivo* for efficacy. Mice were fasted for 12 h and then orally administered with the complexes at 50 U insulin/kg. Ninety minutes after the administration of these particle complexes, all mice (n=5 per group) were restrained and a magnetic field was externally applied to the abdomen of the experimental group. An additional control group received 200 μL water only. The glucose level of each mouse was monitored over time using the One Touch Ultra glucose monitor (Bayer). Blood samples were taken from each animal and analyzed for insulin level using Mercodia ELISA kit. Studies with heparin-containing PolyMP- were similarly conducted. Blood was collected from dosed mice and centrifuged at 10000 rpm in plasma tubes. Plasma was then analyzed for Heparin levels using a factor Xa assay (DiaPharma). Bioavailabilities were calculated by area under curve (AUC) of the glucose, insulin, or heparin blood levels over time. Standards for insulin and glucose bioavailability were direct i.v. administration. I.P. administered heparin served as the standard for heparin bioavailability.

Example 15: Discussion of Magnetically Responsive Polymeric Microparticles for Oral Delivery of Protein Drugs

To incorporate magnetic targeting into an oral drug delivery system, we co-encapsulated magnetite (10-15 nm in diameter as measured by transmission electron microscopy, data not shown) and insulin into polymer microparticles using the double emulsion method. Phase separation of the hydrophobic polymer in the organic phase from the aqueous phase results in random distribution of the magnetite nanocrystals in the polymer matrix and generates magnetically responsive polymeric MPs. The average sizes of MPs containing 0 %, 2% and 5 wt% magnetite are 4.6 μm, 6.4 μm, and 7.2 μm with insulin encapsulation efficiencies of 68%, 78% and 79%, respectively. MPs containing 2-5% magnetite are 40-56% larger than MPs without magnetite. The spherical structures of MPs containing 0-5% magnetite were very well maintained, and the majority of particles observed under SEM show minimum disruption of their spherical structures (Figure 1A-C). When the content of magnetite increases to 10%, however, the spherical structures of the resulting MPs become less stable. The lower stability of the MPs may be due to the weakening of the polymer matrix connections with increased magnetite nanocrystal content (Figure 1D).

The application of an external magnetic field applied to the intestinal area, for example, with a magnetic belt, can slow down the transit of magnetite-containing polymeric particles and extend the residence time of the orally delivered microparticles in the small intestine, which will potentially increase the absorption of protein drugs. PLGA may be used instead of PLA microparticles to increase the release rate of insulin in small intestine. Insulin delivery from PLGA microparticles was significantly greater in the presence of a magnetic field, resulting in a substantially improved hypoglycemic effect in mice, demonstrating that magnetic forces can improve the efficiency of orally delivered protein therapeutics.

The retention of MPs in the small intestine was modeled *in vitro* using a flow system as shown in Figure 7. Silicone tubing with an internal diameter similar to the small intestine of the mouse was used as the model system. To measure the retention of magnetite-containing MPs in the presence of an external field, an aliquot of ^{125}I-insulin co-encapsulated PLA MPs was injected into the flow apparatus. PLA MPs were used in this study to minimize the initial burst release of free ^{125}I-insulin, in order to facilitate the accurate measurement of eluted particles rather than released

insulin. The flow rate of the mobile phase (1 x PBS, pH = 7.4) was maintained at 2.5 ml/min even though the fluid flow rate in the small intestine of mouse is estimated to 0.03 ml/min (17,18). We purposely chose a flow rate much higher than the actual flow rate in the mouse small intestine to counterbalance intestinal contraction forces that cannot be easily modeled and thus were omitted in this study.

The ^{125}I activity of each fraction collected from the *in vitro* flow apparatus was analyzed on a scintillation counter and normalized against the total amount of radioactivity PLA MPs injected through syringe B. In the presence of an external magnetic field, elution of the majority of the MPs without magnetite occurred in a shorter time than for magnetite-containing MPs (Figure 13A). Cumulatively, 66.4% elution of control MPs was observed during the first minute as compared to 14.1% and 4.0% for the MPs containing 2% and 5% of magnetites, respectively (Figure 3B). When control MPs were completely eluted at t = 5 minutes, only 17.1% and 7.7% of MPs containing 2% and 5% of magnetite, respectively, were eluted. This study demonstrated that magnetite encapsulation in PLA MP induced magnetic responsiveness of the particles. The retention of the magnetite-containing MPs under a constant flow rate is inversely proportional to the magnetite content in these MPs.

The use of an external magnetic field to increase the retention of ^{125}I-insulin - magnetite-PLGA MPs *in vivo* was evaluated in mice. After 6 hours, the recovered radioactivity in the small intestine, as a percent of total dose, was 8.75 ± 2.2% and 4.35 ± 1.1% ($P = 0.015$) for the groups restrained in the presence or absence of a magnetic field, respectively. At 12 hours, the amount recovered for the two groups was 3.80 ± 1.1% and 1.55 ± 0.7% ($P = 0.039$), respectively. Thus, the intestinal retention of ^{125}I-insulin-magnetite-PLGA MPs was improved by 101%-145% in the presence of a magnetic field 6-12 hours after oral administration (Figure 3A). The blood radioactivity was higher in the magnet applied groups by 143% and 189% at 6 and 12 hours, respectively. The serum radioactivity decreased more than 50% from 6h to 12h in both groups (data not shown). Thus, the greater quantity of ^{125}I-insulin recovered in the small intestine for the magnet-applied group compared to the control group was due to increased residence time and not increased absorption. We further evaluated the efficacy of Humulin R encapsulated, magnetite containing PLGA MPs. A single administration of 50 unit/kg of Humulin R-magnetite-PLGA microparticles to fasted mice resulted in 66% reduction of blood glucose level in the presence of

external magnetic field at 12 h, compared to 27% reduction in the absence of magnetic field (Figure 3B).

We hypothesized that extending the residence time of insulin containing microparticles in the small intestine would improve pharmacological efficacy, since more insulin can be released and then absorbed. This hypothesis was tested by monitoring glucose levels in animals. After an initial increase in blood glucose in response to stress associated with the oral gavage method, the mice became hypoglycemic due to the treatment. Our studies indicated that magnetically assisted delivery of insulin-containing microparticles decrease glucose levels for at long as 20 hours. A single oral administration of 100 U/kg of Humulin R-magnetite-PLGA MPs to fasted mice resulted in 43.5% reduction of blood glucose concentration in the presence of an externally applied magnetic field after 4 hours. Interestingly, this blood glucose was maintained at this level for at least additional 16 hours (42.8% and 43.2% of reduction of blood glucose concentration at $t = 12$ and 20 h, respectively). Mice gavaged a single dose of 100 U/kg of Humulin R-magnetite-PLGA MPs in the absence of a magnetic field showed a glucose reduction of 18.6% at 12 hours (Figure 14). The average decreased AUCs are $763 \pm 112\% \cdot h$ and $218 \pm 127\% \cdot h$ ($P < 0.01$) for the groups restrained in the presence and absence of an external magnetic field, respectively.

Intravenous (i.v.) administered Humulin R at a dose of 0.5 U/kg served as a standard for bioavailability. The highest glucose reduction (46%) was observed 1h after i.v. dosing, and this time for glucose depression lasted for about 4-5 hours (Figure 14). The average AUC of the i.v. group was $120.9 \cdot h \pm 45.2\%$. According to equation (1), the bioavailability was calculated to be $2.77 \pm 0.46\%$ and $0.66 \pm 0.56\%$ ($p < 0.01$) for the groups restrained in the presence and absence of an external magnet, respectively. Application of a magnetic field to the mice orally dosed with magnetically responsive, insulin PLGA MPs led to an increase of bioavailability by 420%.

The magnetic-induced increment of bioavailability was confirmed by direct analysis of serum insulin levels (Figure 15). Magnetically assisted delivery of insulin and magnetite co-encapsulated microparticles provided the highest level of insulin concentration of 68 mU/L at $t = 2$ h, which gradually decreased to 18.2 mU/L at $t = 20$ h. As a comparison, the control group without the external magnet reached the highest

level of insulin concentration of 31 mU/L at t = 1 h, which gradually decreased to 7.1 mU/L at t = 20 h (Figure 15A). Intravenous (i.v.) administered Humulin R at a dose of 2 U/kg served as a standard for bioavailability. The highest concentration of insulin (3.2×10^3 mU/L) was observed 6 minutes after administration, and the insulin level rapidly decreased to 14 mU/L at 2h (Figure 15B). Bioavailability calculated based on the AUC derived from insulin concentration measurement are $0.87 \pm 0.29\%$ and $0.30 \pm 0.06\%$ ($p < 0.03$) for the groups restrained in the presence and absence of an external magnet, respectively.

The bioavailabilities obtained from serum insulin measurement are roughly 30-45% of the bioavailability obtained from blood glucose measurements. This difference is presumably due to the different detection techniques. Previous work indicated that 50% of insulin absorbed from the small intestine will be entrapped or metabolized in the liver and not released back into the blood (first pass effect) (15). However, this insulin can still regulate glucose level to some degree but is not detectable in serum insulin measurements. Similar comparisons between glucose and insulin bioavailabilites due to the difference of detection techniques was also reported by others (15).

After demonstrating the efficacy of this approach, we investigated the biocompatibility of the magnetite-PLGA MPs in gastrointestinal tissue as well as toxicity in other organ systems. Microscopic analysis of histological tissue sections reveals no apparent signs of increased acute inflammation, leukocyte infiltration, tissue edema or necrosis. When comparing tissue sections derived from animals administered with MPs versus controls, small intestine tissue section analysis does not show enhanced migration of macrophages or increased numbers of neutrophils and lymphocytes. In addition, tissue sections from mice administered with the magnetite-PLGA MPs did not exhibit the formation of an inflammatory exudate which is often observed as a response to an acute tissue insult (Figure 16A). A similar lack of toxicity is exhibited in the other tissue sections as well, namely liver (Figure 16B), spleen (Figure 16C) and kidney (Figure 16D). Examination of tissue sections stained for colloidal iron showed no detectable accumulation of magnetites (positive control slide provided in inset). After 24 hours, the magnetites were no longer present in the small intestine (Figure 17A) and were not observed in the liver (Figure 17B). Based on this histological analysis of various tissues from the dosed animals and controls,

we conclude that administration of the magnetite particles is accompanied with little if any acute toxicity in these organ systems. Further, the magnetites do not seem to be absorbed after administration.

2. Magnetically responsive oral delivery via charge interaction

Although the magnetite encapsulated system can substantially improve the retention of protein delivery particles, they have two fundamental drawbacks: relative weak magnetic responsiveness and potential toxicity due to possible absorption of nanometer sized magnetite. Absorption of magnetite via gastrointestinal tract into circulatory system may lead to the concerns regarding systemic toxicity, especially during the treatment of chronic diseases, although there are suggestions that liver can metabolize iron and our preliminary studies indicated low or no absorption of nano-sized magnetite.

One way to minimize potential intestinal absorption of magnetite is to increase its size in excess of the threshold for epithelial membrane penetration. When magnetites are sufficiently large (e.g., > 5 μm), they tend to stay in the gastrointestinal tract (GIT) after oral administration and are eventually eliminated. In addition, the micrometer-sized magnetites also have stronger magnetic responsiveness. The larger mass allows the micromagnetites to apply more retentive force to the polymer MPs. An exemplary design is illustrated in Figure 18. We use surface-charged magnetic microparticles (MagMPs) to immobilize PLGA-insulin nanoparticles MPs (PolyMPs) bearing opposite surface charges. When this complex is orally administered and an external magnetic field is applied to the intestinal area, the PolyMPs are likely immobilized in the intestine through the charge interaction with magnetic MPs.

The PolyMP with COOH end groups yielding a negatively charged surface (PolyMP-). The magnetic particles functionalized with $-NH_2$ groups gave a positively charged surface (MagMP+). Mixing of PolyMP,- with MagMP+ led to their quick and strong association (Fig. 5 and 19). SEM analysis displayed the strong interaction between irregularly shaped MagMP+ and spherical PolyMP- (Fig. 5A). BioMag-NH_2 (6 μm) can immobilize several MPs to form simple complexes as shown in Figure 5A or large aggregates as shown in Figure 5B. When a magnet (1" (L) x 1" (W) x 0.5" (H) NdFeB plate magnet) is applied to a mixture of MagMP and PolyMP bearing either oppositely or identically surface charges, only the former mixture (both MagMP+ and PolyMP-) is immobilized and precipitated instantly (low-

left, Fig. 19). In the mixture having particles with identical surface charge, only the MagMP precipitated while the PolyMP remain as colloidal suspension (low-right, Fig. 19). This assay clearly indicates surface charge is responsible for the sedimentation of PolyMP indirectly through the interaction of MagMP and external magnetic field.

The interaction is strongly correlated to the relative surface areas of MagMP and PolyMP. When the PolyMP- with diameters 3-5μm is coupled to Bio MagNH$_2$, plus (Polysciences, Inc), a commercially available MagMP+ with average diameter of 1 μm, a strong interaction between PolyMP- and MagMP+ were achieved at a mass ratio 15 (Fig. 20). More than 95% of PolyMP- still can be precipitated and retained by magnetic force through charge interactions with MagMP (Fig. 20). This observation implies that the exemplary PolyMP delivery system described above can be further enlarged to a few tens or hundreds of microns (i.e. to further reduce their surface size) to minimize the amount of MagMP introduced for effective coupling. Once coupled, the particles were not separable in physiological conditions ranging from pH 2.5 to 8.3 (Fig. 21). This pH range covers the acidic environment of the stomach to the slightly basic pH of the lower small intestine.

An *in vitro* flow study was conduced to examine both the response of the PolyMP-/MagMP+ complexes to an external magnetic field and the potential de-coupling of the particles. Eluted fractions in super-physiological flow conditions mimicking the small intestine of a mouse model showed almost no release of insulin or insulin-containing particles (Fig. 8a, comparison of eluted ^{125}I Insulin to no magnetic field control). Thus, as the magnetically immobilized PLGA particles degrade, insulin released into the small intestine can be absorbed across the epithelial wall and can enter the bloodstream in a pharmacologically active form. Previous studies have shown insulin released in the small intestine is absorbed in its bioactive form, and the absorption is proportional to the local concentration gradient of insulin. Our *in vivo* study showed excellent immobilization of PolyMP-/MagMP+ by external field (Fig. 22b). 32.5% of administered PolyMPs- are recovered from small intestine alone 6h after dosing and application of an external magnet field, as compared to only 5.4% of total recovered dose in the absence of magnetic field. The enhanced retention by the using magnetic field was further evidenced by a histological analysis of small intestines of mice (Fig. 23a and 23b) treated with Rhodamine-dextran encapsulated PolyMP-/MagMP+ with a magnet similarly applied to the abdominal area of the mice.

Additionally, we monitored long-term efficacy and bioavailability of the complexes by administering human insulin in coupled PLGA PolyMP to fasted Balb/c mice. Mice orally dosed with Insulin PolyMP-/MagMP+ at 100 U /kg with abdominally applied magnetic fields exhibited reduced glucose levels for as long as 36 hours (Figure 15a), corresponding to a bioavailability of 5.11% ± 1.24 calculated by comparing the Area-Under-the-Curve with the tail vein injection intravenous group. The bioavailability reduced to 0.82% ± 0.37 in control oral group without external magnetic field (Figure 24A). The blood insulin levels, as analyzed by insulin ELISA assays, stayed elevated in the group with magnetic field for about 20 hours (data not shown). Insulin has a very short circulating half-life, about 28 minutes, and therefore insulin administered into the blood will reduce glucose levels for a short time. Glucose levels will usually return to normal levels within 3-4 hours after injection of insulin via intraperitoneally or intravenously. Thus, any long-term glucose reduction should be the result of consistent release and absorption of insulin in the small intestine. Oral administration of PolyMP-/MagMP+ with subsequent application of a magnetic field provides the possibility of maintaining hypoglycemia for about two days with a single dose.

We also tested the bioavailability of orally delivery of low molecular weight heparin using the same strategy. Low molecular weight heparin exhibited greater ease of absorption into the blood, most likely due to its small size compared to insulin. The heparin level (obtained by using a factor Xa assay) remained elevated in the group treated similarly with an external magnetic field (Fig. 24B), corresponding to a bioavailability of 4.05 ± 0.16. In the absence of magnetic field, a bioavailability of 2.89 ± 0.32 was obtained (Fig. 24B).

While we used insulin and heparin as model drugs in this study, the strategy allowing long-term retention of PolyMP in the intestine can be used for delivery of other therapeutics, especially for therapeutics having great intestinal permeability such as small molecules or for therapeutics targeting GIT diseases. Histological analysis for toxicity did not show any abnormal physiological change in intestines (Fig. 24C) as well as other organs such as liver, spleen, heart, and kidney (data not shown) for the groups applied magnetic field.

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Other embodiments of the invention will be apparent to those skilled in the art from a consideration of the specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

What is claimed is:

1. A drug delivery composition, comprising:
a magnetic particle;
a pharmaceutically active agent associated with the particle; and
an ingestible carrier containing the particle.

2. The composition of claim 1, wherein the magnetic particle has a dimension between 5 nm and 15 mm.

3. The composition of claim 1, wherein the magnetic particle has a dimension greater than 5 microns.

4. The composition of claim 1, wherein the magnetic particle is functionalized with one or more of biotin, streptavidin, protein A, protein G, an oligonucleotide, an amine, a carboxylate, and an organosilane.

5. The composition of claim 1, wherein the pharmaceutically active agent is associated with the particle by a covalent or a non-covalent interaction.

6. The composition of claim 1, further comprising a plurality of magnetic particles, wherein a biologically active agent is associated with each particle.

7. The composition of claim 1, wherein the magnetic particle and the pharmaceutically active agent are co-encapsulated within a polymer particle.

8. The composition of claim 1, wherein the pharmaceutically active agent is encapsulated in polymeric particles, and wherein the magnetic particle is associated with at least one of the polymeric particles.

9. The composition of claim 8, wherein the polymeric particles comprise a synthetic polymer or a non-synthetic polymer.

10. The composition of claim 9, wherein the polymer is functionalized with one or more of a charged or chargable chemical group, hydroxyl, amine, carboxy, maleimide, thiol, N-hydroxy-succinimide (NHS) esters, alkyne, azide, biotin, streptavidin, polyhistidine, poly(ethylene glycol), and poly(propylene glycol).

11. The composition of claim 8, wherein the composition comprises a plurality of polymeric particles associated with at least one magnetic particle.

12. The composition of claim 8, wherein the composition comprises a plurality of polymeric particles and a plurality of magnetic particles associated with one another in an extended network.

13. The composition of claim 1, wherein the pharmaceutically active agent is associated with a polymer, and wherein the polymer is associated with the magnetic particle.

14. The composition of claim 1, wherein the pharmaceutically active agent is insulin.

15. A method of delivering a pharmaceutically active agent, comprising:
providing the agent, wherein the agent is associated with a magnetic particle; and
orally administering the agent to a patient in need thereof.

16. The method of claim 15, further comprising applying a magnetic field to at least a portion of the lower gastrointestinal tract of the patient.

17. The method of claim 15, wherein the magnetic particle passes through the intestinal wall to deliver the pharmaceutically active agent to the bloodstream.

18. The method of claim 15, wherein the magnetic particle retains the pharmaceutically active agent in the intestine until it is able to pass through the intestinal wall into the bloodstream, wherein the magnetic particle does not pass through the intestinal wall.

19. The method of claim 15, further comprising using a magnet to retain the magnetic particles at a tissue site that is not a target tissue site of the pharmaceutically active agent.

20. The method of claim 19, wherein the magnet provides a magnetic field of about 1 Gauss to about 10 Gauss.

21. The method of claim 19,wherein the magnet provides a magnetic field of about 10 Gauss to about 100 Gauss.

22. The method of claim 19,wherein the magnet provides a magnetic field of about 100 Gauss to about 1000 Gauss.

23. The method of claim 19,wherein the magnet provides a magnetic field of about 1000 Gauss to about 1 Tesla.

24. The method of claim 19,wherein the magnet provides a magnetic field of greater than about 1 T.

25. The method of claim 19,wherein the magnet is disposed on a garment.

26. The method of claim 19,wherein the magnet is a plurality of magnets disposed on a garment.

27. The method of claim 19,wherein the garment is a belt.

28. A method of preparing a pharmaceutical composition, comprising:
associating a pharmaceutically active agent with a polymer to form an agent-polymer aggregate; and
associating the agent-polymer aggregate with a magnetic particle.

29. The method of claim 28, wherein the magnetic particle is functionalized with one or more of biotin, streptavidin, protein A, protein G, an oligonucleotide, an amine, a carboxylate, and an organosilane.

30. The method of claim 28, wherein the agent-polymer aggregate is associated with the magnetic particle via a covalent interaction or a non-covalent interaction.

31. The method of claim 28, wherein the agent is encapsulated in the polymer to form the agent-polymer aggregate.

32. The method of claim 28, wherein the polymeric particles comprise a synthetic polymer or a non-synthetic polymer.

33. The method of claim 28, wherein the polymer is functionalized with one or more of a charged or chargable chemical group, hydroxyl, amine, carboxy, maleimide, thiol, N-hydroxy-succinimide (NHS) esters, alkyne, azide, biotin, streptavidin, polyhistidine, poly(ethylene glycol), and poly(propylene glycol).

34. The method of claim 31, wherein the agent-polymer aggregate has a predetermined surface charge, and wherein the agent-polymer aggregate is associated with the magnetic particle via a charge interaction.

35. The method of claim 31, wherein a plurality of agent-polymer aggregates are associated with the magnetic particle via charge interactions.

36. The method of claim 28, wherein associating the agent-polymer aggregate comprises encapsulating the agent and at least one a magnetic particle in the polymer.

37. A kit for delivering a pharmaceutically active agent, comprising:
a pharmaceutically acceptable carrier containing a plurality of magnetic particles having the agent associated with them; and
a magnet sufficiently strong to hinder the passage of the particles away from a tissue site when the magnet is placed in the vicinity of the tissue site.

38. The kit of claim 37, wherein the magnet provides a magnetic field of about 1 Gauss to about 10 Gauss.

39. The kit of claim 37, wherein the magnet provides a magnetic field of about 10 Gauss to about 100 Gauss.

40. The kit of claim 37, wherein the magnet provides a magnetic field of about 100 Gauss to about 1000 Gauss.

41. The kit of claim 37, wherein the magnet provides a magnetic field of about 1000 Gauss to about 1 Tesla.

42. The kit of claim 37, wherein the magnet provides a magnetic field of greater than about 1 T.

43. The kit of claim 37, wherein the magnet is disposed on a garment.

44. The kit of claim 37, wherein the magnet is a plurality of magnets disposed on a garment.

45. The kit of claim 37, wherein the garment is a belt.

46. A composition, comprising:
a plurality of magnetic particles;
a pharmaceutically active agent associated with the particles, and
an ingestible carrier within which the plurality of magnetic particles are distributed.

47. The composition of claim 46, wherein the magnetic particles have a dimension between 5 nm and 100 mm.

48. The composition of claim 46, wherein the magnetic particles have a dimension greater than 5 microns.

49. The composition of claim 46, wherein the magnetic particles and the pharmaceutically active agent are co-encapsulated within at least one polymer particle.

50. The composition of claim 46, wherein the pharmaceutically active agent is encapsulated in polymeric particles, and wherein the magnetic particles are associated with the polymeric particles.

51. The composition of claim 50, wherein the magnetic particles have a predetermined surface charge, and wherein the polymeric particles have the opposite surface charge, and wherein the magnetic particles and the polymeric particles are associated by charge interactions.

52. The composition of claim 51, wherein the association of the magnetic particles and the polymeric particles is stable with respect to dissociation from about pH 2.5 to about pH 8.3.

53. The composition of claim 51, wherein the plurality of polymeric particles and the plurality of magnetic particles are associated with one another in an extended network.

54. The composition of claim 50, wherein the polymeric particles comprise a synthetic polymer or a non-synthetic polymer.

55. The composition of claim 50, wherein the polymer is functionalized with one or more of a charged or chargable chemical group, hydroxyl, amine, carboxy, maleimide, thiol, N-hydroxy-succinimide (NHS) esters, alkyne, azide, biotin, streptavidin, polyhistidine, poly(ethylene glycol), and poly(propylene glycol).

56. The composition of claim 46, wherein the pharmaceutically active agent is associated with a polymer, and wherein the polymer is associated with the magnetic particles.

57. The composition of claim 50, wherein the pharmaceutically active agent is insulin.

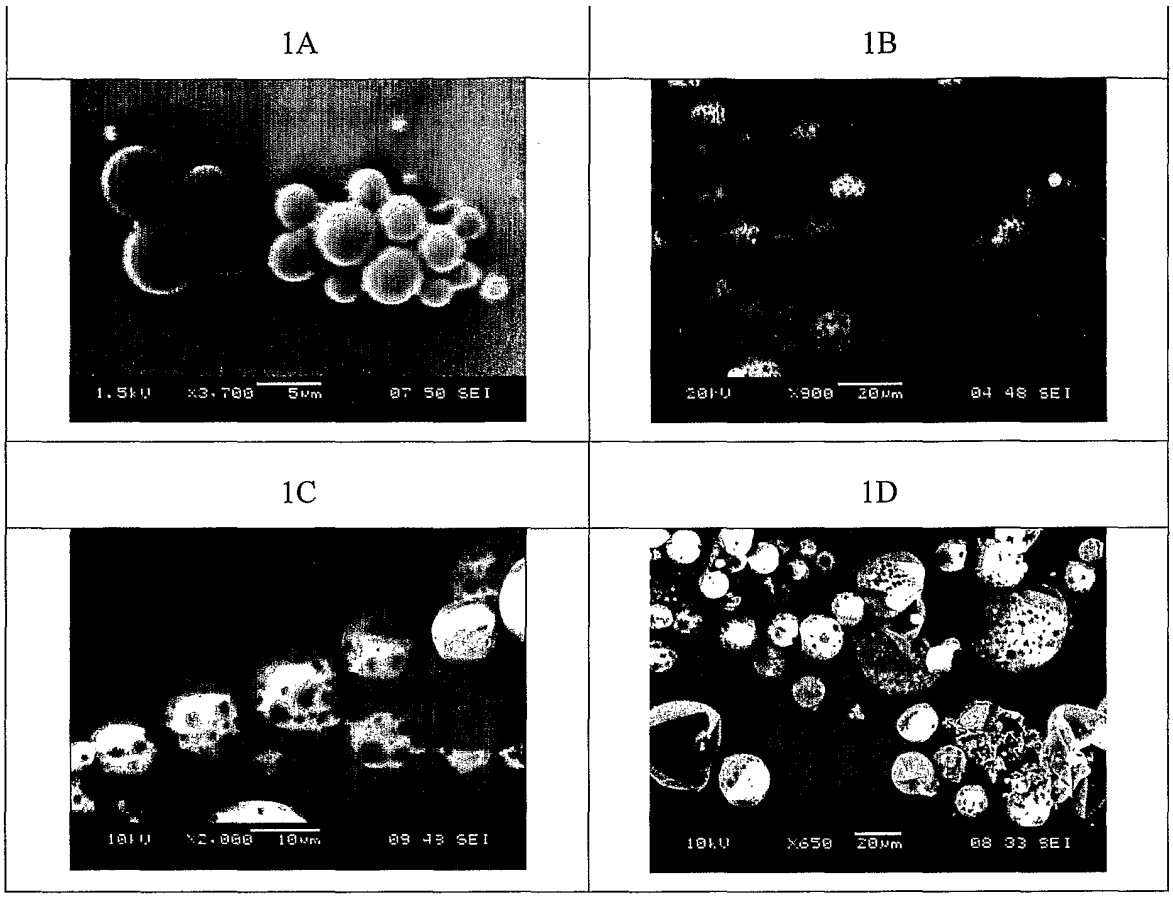


Figure 1

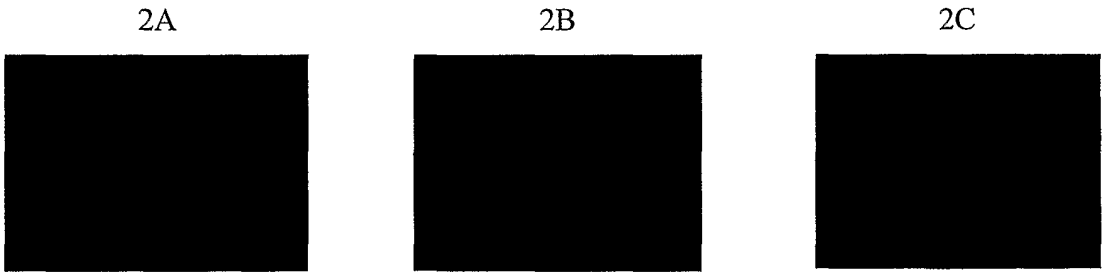


Figure 2

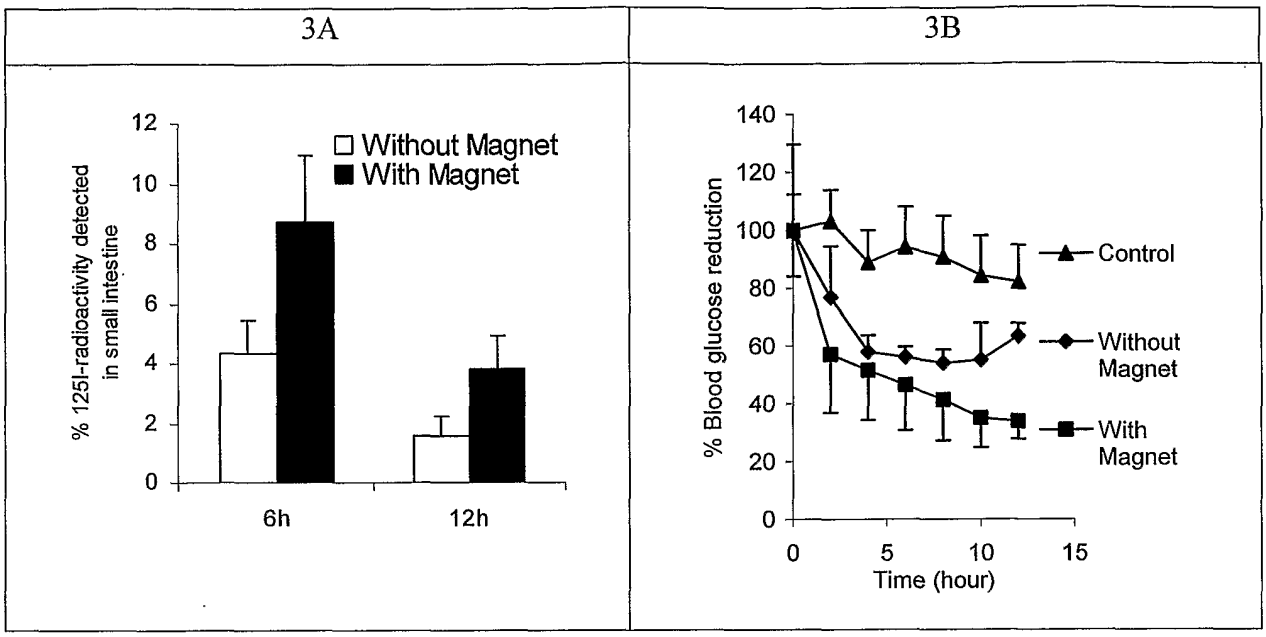


Figure 3

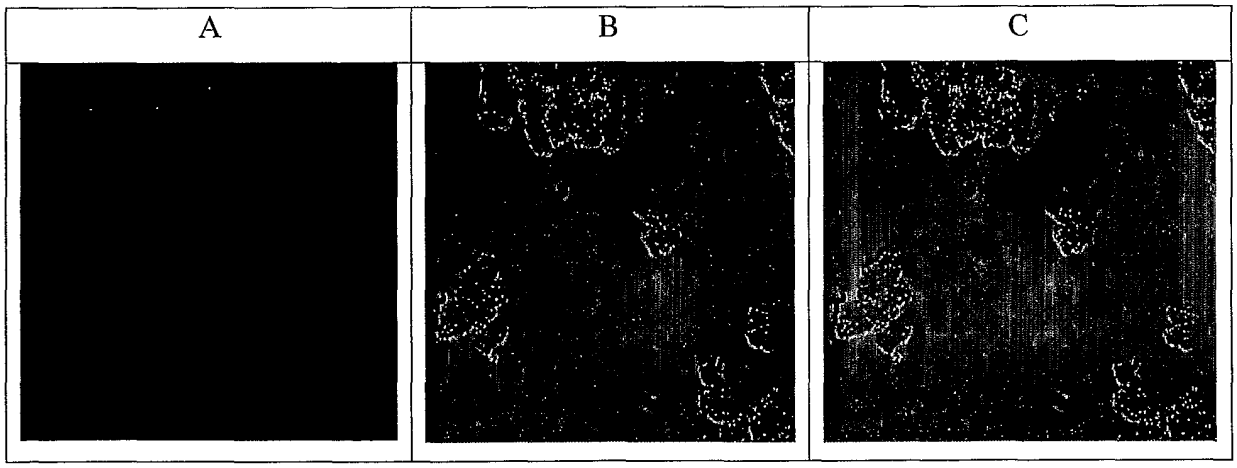


Figure 4

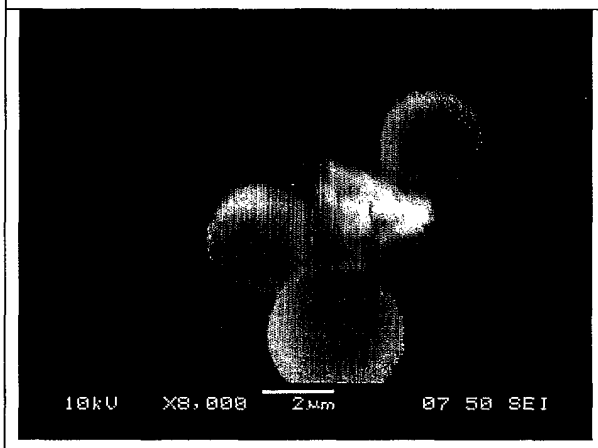
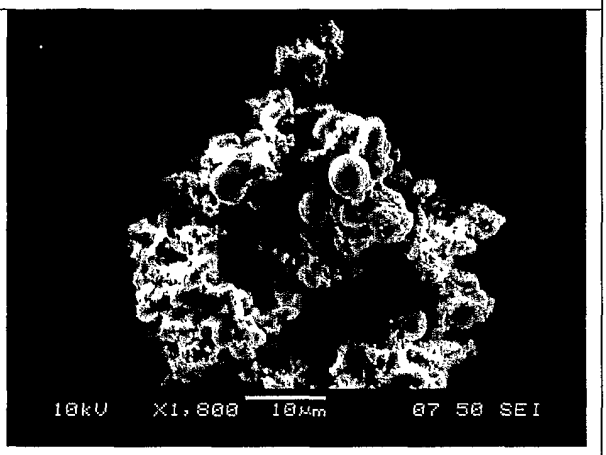
A. BioMag-NH_2 (6 μm)/PLA-COOH MP-1	B. BioMag-NH_2 (6 μm)/PLA-COOH MP-2
	

Figure 5

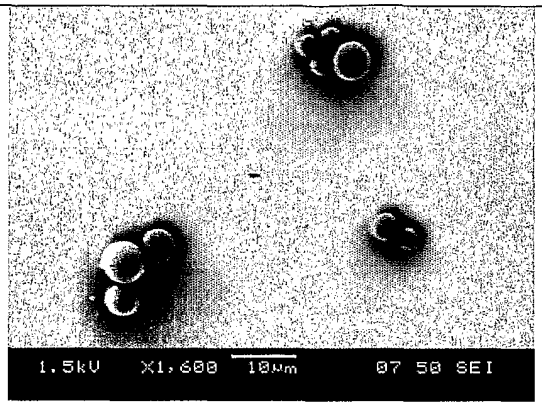
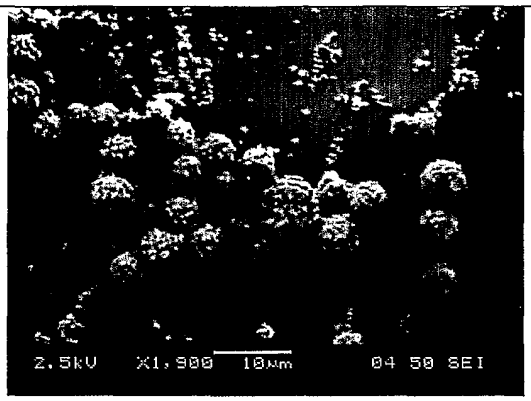
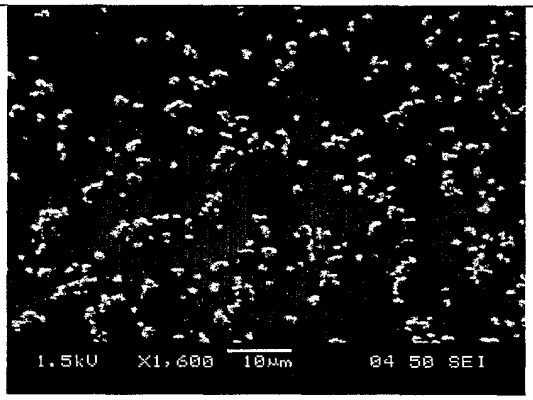
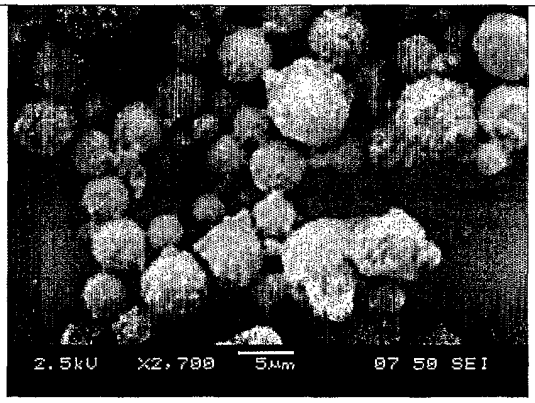
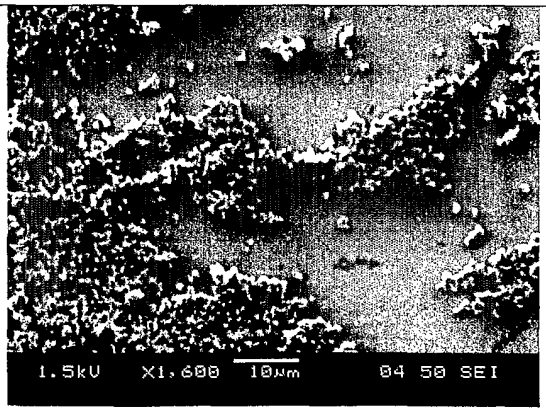
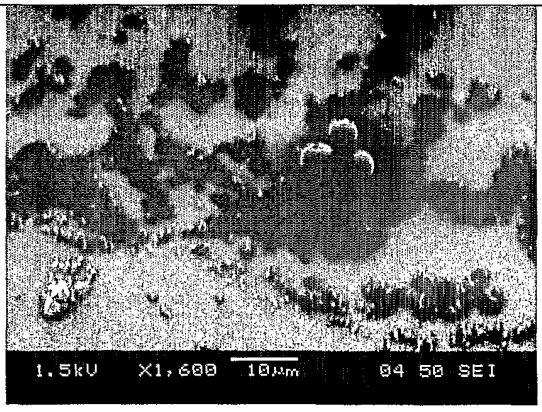
A: PLA-COOH MP	D: BioMag-COOH/ Chitosan-PLA-MP
	
B: BioMag-NH_2 (1 µm)	E: BioMag-NH_2/PLA-COOH MP
	
C: BioMag-COOH (1 µm)	F: BioMag-COOH/ PLA-COOH MP
	

Figure 6

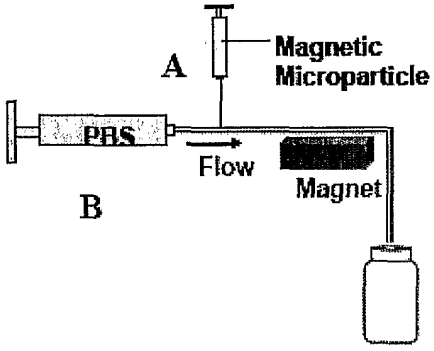


Figure 7

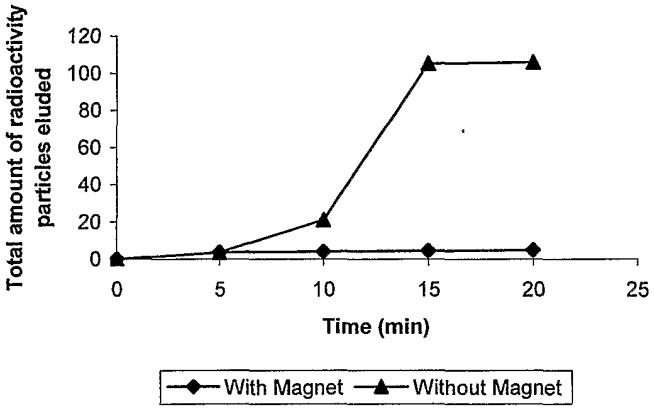


Figure 8

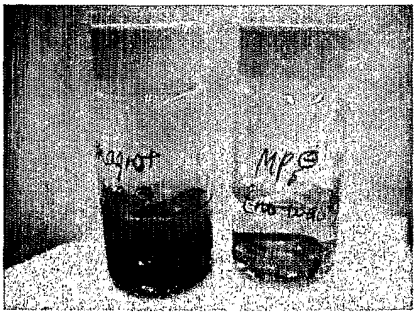


Figure 9

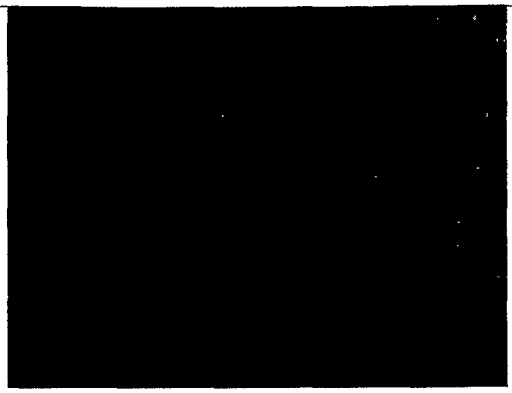
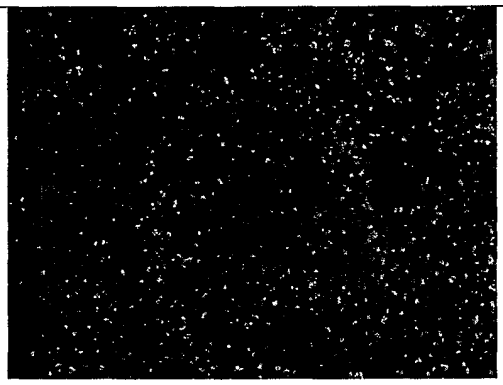
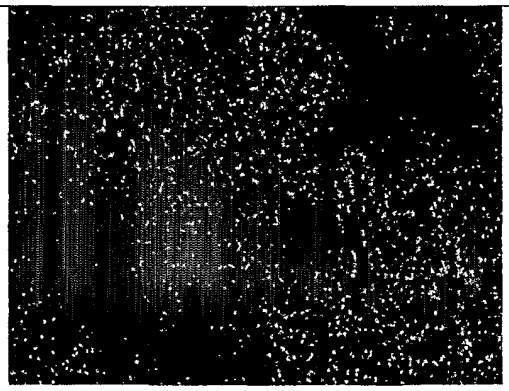
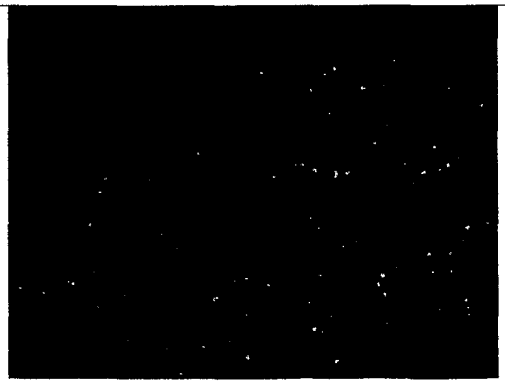
A: Overlay Chitosan-MP/BioMag-COOH without magnet	B: Overlay MP/BioMag-NH_2 without magnet
	
C: Overlay MP/BioMag-NH_2 with magnet (image 1)	D: Overlay MP/BioMag-NH_2 with magnet (image 2)
	
E. Overlay BioMag-COOH/Chitosan-MP with magnet (image 1)	F: Overlay BioMag-COOH/Chitosan-MP with magnet (image 2)
	

Figure 10

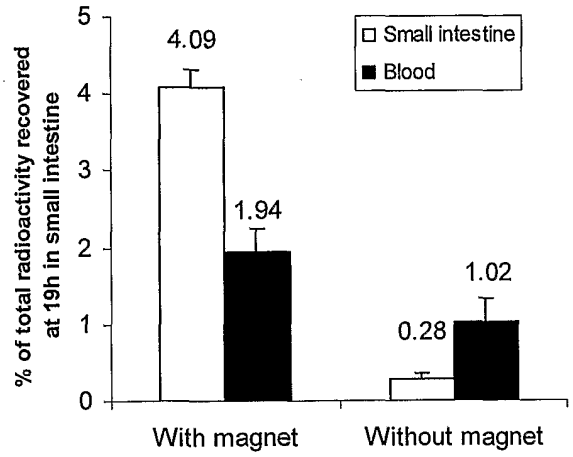


Figure 11

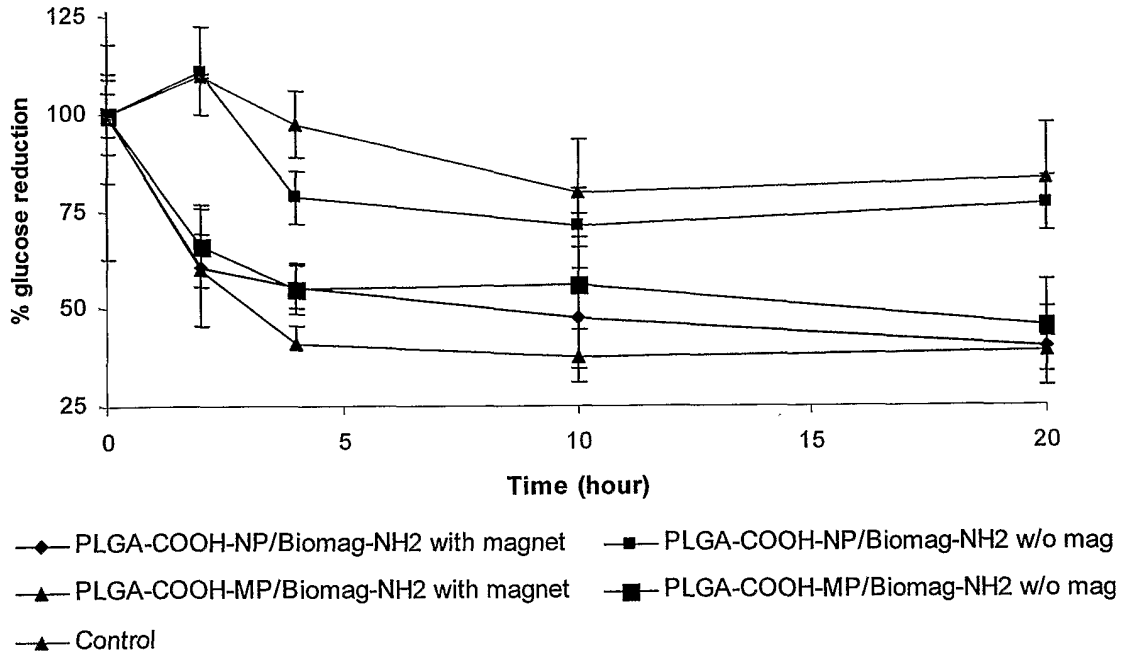


Figure 12

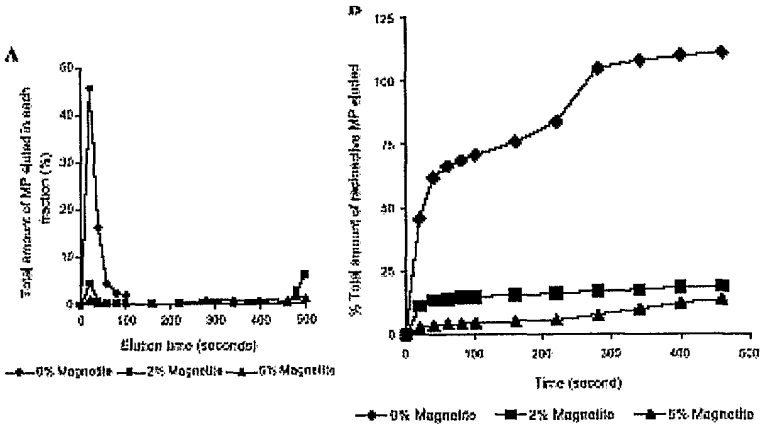


Figure 13

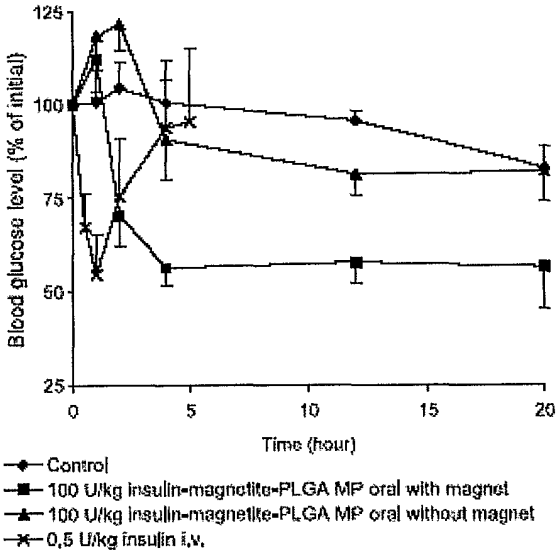


Figure 14

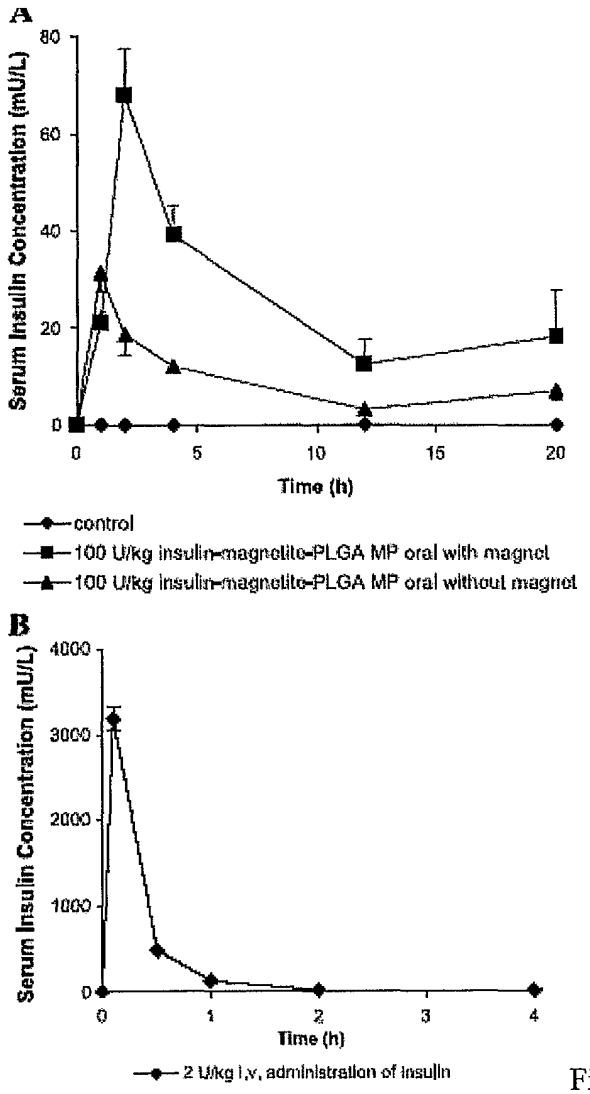

A
Serum Insulin Concentration (mU/L)
80
60
40
20
0
0
5
10
15
20
Time (h)
control
100 U/kg insulin-magnetite-PLGA MP oral with magnet
100 U/kg insulin-magnetite-PLGA MP oral without magnet
B
Serum Insulin Concentration (mU/L)
4000
3000
2000
1000
0
0
1
2
3
4
Time (h)
2 U/kg I.v. administration of insulin

Figure 15

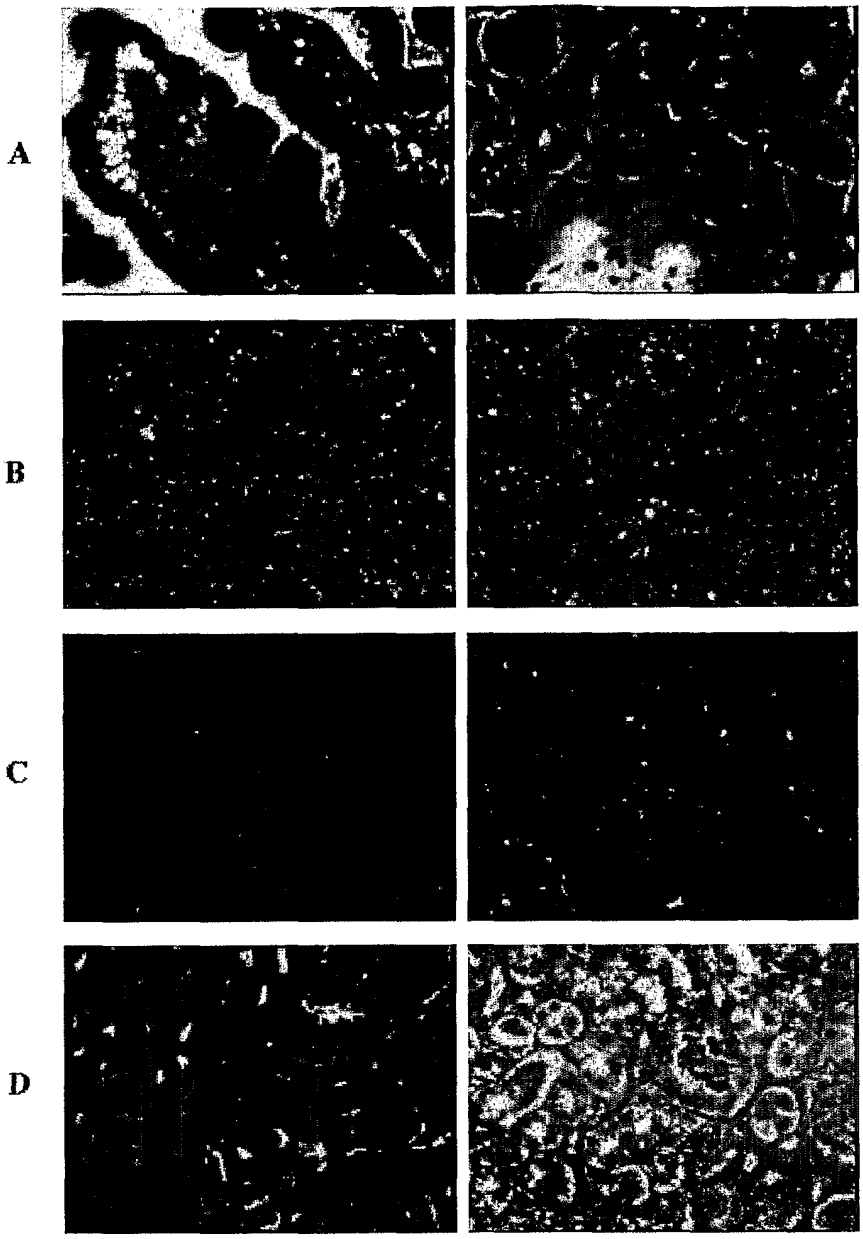


Figure 16

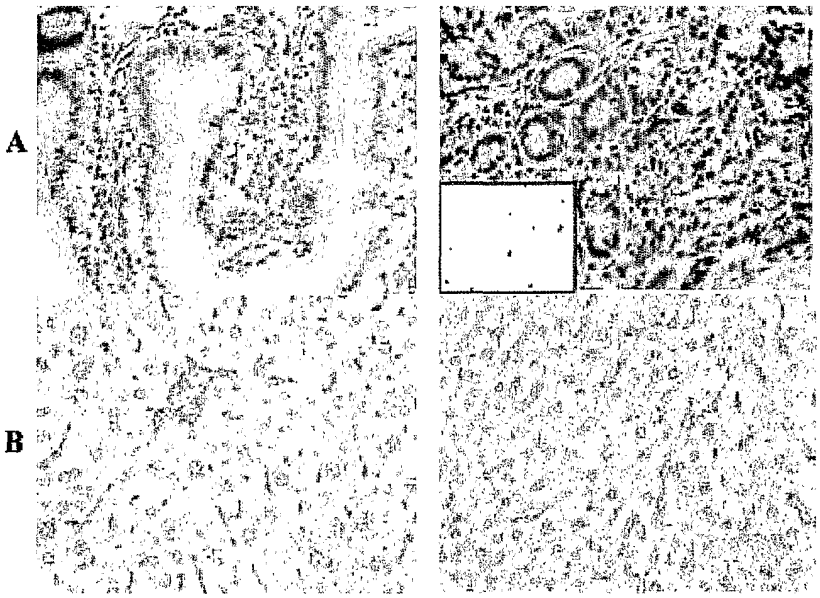


Figure 17

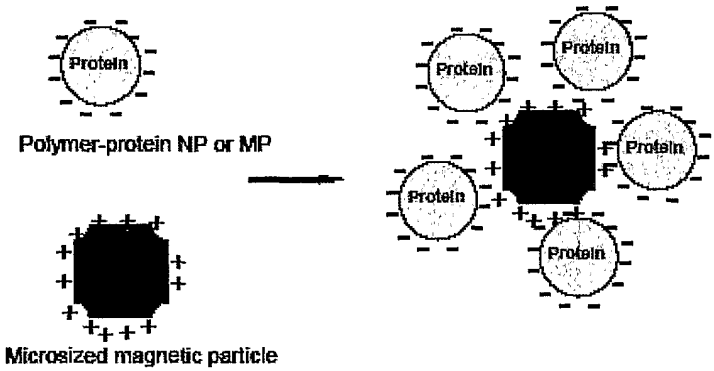


Figure 18

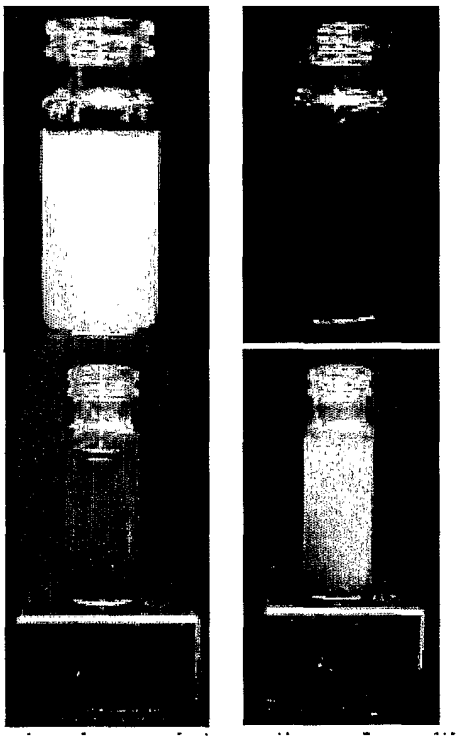


Figure 19

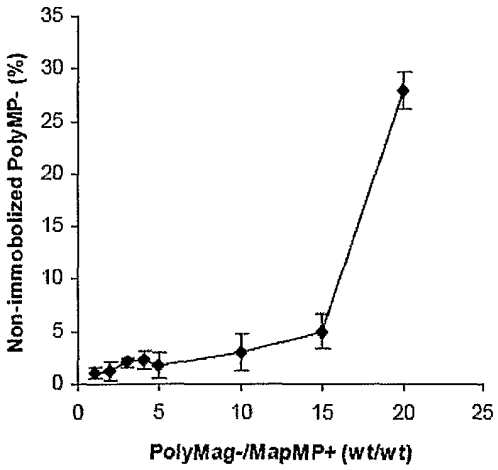


Figure 20

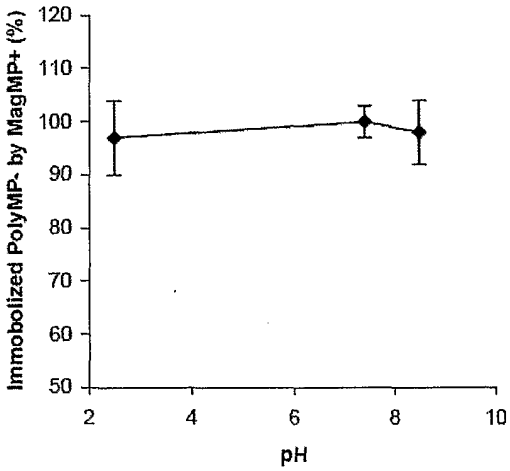


Figure 21

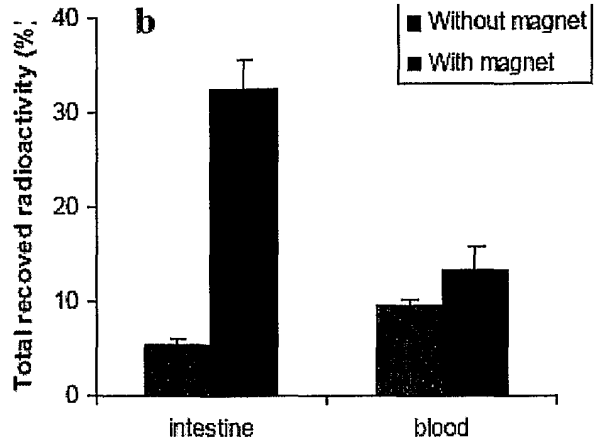


Figure 22

Figure 23A

Figure 23B

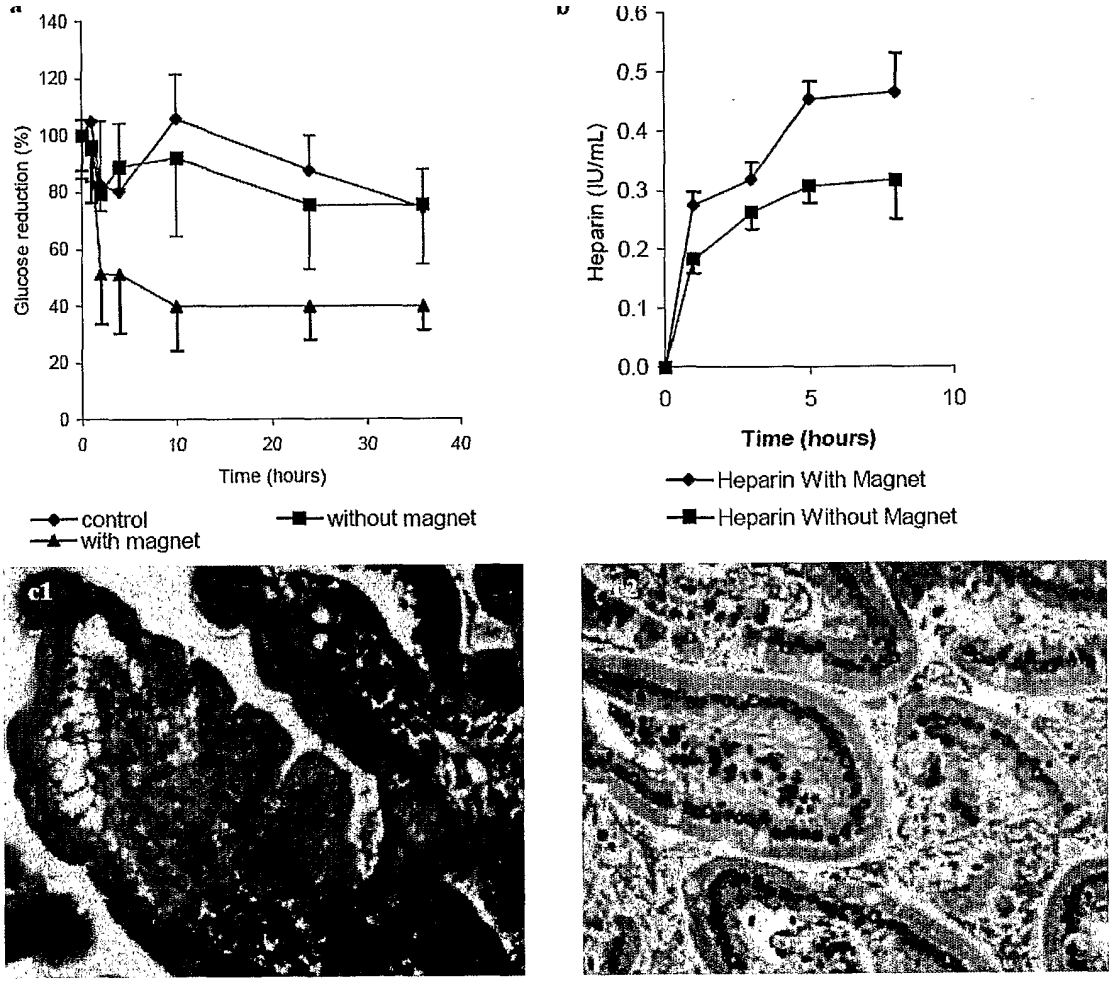

a
Glucose reduction (%)
Time (hours)
control
with magnet
without magnet
b
Heparin (IU/mL)
Time (hours)
Heparin With Magnet
Heparin Without Magnet
c1
c2

Figure 24