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<p>(54) Title: LOCAL DELIVERY OF MEDICATIONS TO THE HEART</p>		
<p>(57) Abstract</p> <p>Microparticle compositions are provided containing pharmacological agents effective when locally released at the heart. They are introduced into the bloodstream and their location is monitored by ultrasound or other means. The microparticles are ruptured with ultrasound energy at a predetermined ultrasound condition when such energy is directed locally at the region of the heart.</p>		

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LOCAL DELIVERY OF MEDICATIONS TO THE HEART

Cross Reference To Related Applications

The priority of Provisional Application No. 60/098,975, filed September 2,
5 1998 is claimed and its content is incorporated by reference herein in its entirety.

Summary of the Invention

This invention relates to the use of drug containing microspheres with
acoustical characteristics that allow the ultrasound release of drugs for treatment of
10 the heart. In particular, the invention relates to specific microparticle characteristics
in combination with specific types of cardiac drugs for ultrasound mediated therapy.

Description of the Preferred Embodiments

As used herein the term microparticles is intended to include microcapsules,
15 microspheres and microbubbles which are hollow particles enclosing a core filled
with a gas. It is not necessary for the microparticles to be precisely spherical
although they generally will be spherical and described as having average diameters.
If the microparticles are not spherical, then the diameters are referred to or linked to
the diameter of a corresponding spherical microparticle having the same mass and
20 enclosing approximately the same volume of interior space as a non-spherical
microparticle.

The microparticles are preferred to have an extended circulatory half-life as
compared to bolus injections of free drug. In order to treat the heart for several
minutes, the microparticles will necessarily need to recirculate back to the heart. The
25 microparticles will need to avoid biological clearance and degradation in order to
recirculate and enable drug treatment for several minutes to hours from a single bolus
injection. Alternatively, the microparticles may be constantly administered by an
infusion pump.

The types of agents to be released by the microparticles are typically
30 cardiovascular drugs with short circulatory half-lives that affect the cardiac tissues,
vasculature and endothelium to protect and treat the heart. Drugs which target

platelets and white cells which may plug the microvasculature of the heart after a heart attack are also useful for local cardiac delivery. A third type of drug useful for local delivery is one for which a local effect is required but where the systemic effects of the drug would be detrimental. These are typically drugs with high toxicity, for example, locally administered potent vasodilators which would increase blood flow to hypoxic tissue, but if delivered systemically would cause a dangerous drop in blood pressure. Suitable drugs include fibrinolytic agents such as tissue plasminogen activator, streptokinase, urokinase, and their derivatives, vasodilators such as verapamil, multifunctional agents such as adenosine, adenosine agonists, adenosine monophosphate, adenosine diphosphate, adenosine triphosphate, and their derivatives, white cell or platelet acting agents such as GPIIb/IIIa antagonists, energy conserving agents such as calcium channel blockers, magnesium and beta blockers, endothelium acting agents such as nitric oxide, nitric oxide donors, nitrates, and their derivatives, free-radical scavenging agents, agents which affect ventricular remodeling such as ACE inhibitors and angiogenic agents, agents that limit ischemic or reperfusion injury to the heart, and agents to limit restenosis of coronary arteries after balloon angioplasty or stenting.

In addition to therapeutic agents delivered locally to the heart, the use of vasodilators in the microparticles will have enhanced diagnostic application. Vasodilators are used in cardiology to assess the coronary blood flow reserve by comparing blood flow in heart with and without the maximal vasodilation by the pharmacological agent. Coronary blood flow reserve correlates well with patient prognosis since the reserve capacity enables the myocardium to remain viable during a heart attack. Adenosine and other vasodilators are used during interventional cardiology and nuclear imaging to determine coronary reserve. A microparticle which contains gas and a vasodilator will be useful in echocardiography to examine the myocardium under normal conditions, and then trigger to release vasodilator by the ultrasound beam conditions to stimulate local vasodilation. The coronary blood flow reserve may be estimated non-invasively using ultrasound imaging by the extent of hyperemia of the myocardium, Doppler regional flow, or by other well known methods of characterizing the ultrasound imaging data.

It is advantageous to use the microparticles since the half-life of the drug

incorporated into the microparticle may be longer than the half-life of the drug in the circulatory system if administered without the microparticles. This gives a prolonged effect of the drug compared to the effect when the drug is administered alone.

The microparticles according to the present invention have a bi-layered shell.

5 The outer layer of the shell will be a biologically compatible material or biomaterial since it defines the surface which will be exposed to the blood and tissues within the body. The inner layer of the shell will be a biodegradable polymer, which may be a synthetic polymer, which may be tailored to provide the desired mechanical and acoustic properties to the shell or provide drug delivery properties. The

10 microparticles will contain gas, typically air or nitrogen, and for drug delivery purposes, a drug incorporated into the microparticle. To make the microparticles rupturable by a low intensity ultrasound energy, they must contain a gas to allow acoustic coupling and particle oscillation. Microparticles are constructed herein such that the majority of those prepared in a composition will have diameters within the

15 range of about one to ten microns in order to pass through the capillary system of the body.

Since the microparticles have an outer and inner layer, the layers can be tailored to serve different functions. The outer shell which is exposed to the blood and tissues serves as the biological interface between the microparticles and the body.

20 Thus it will be made of a biocompatible material which is typically amphiphilic, that is, has both hydrophobic and hydrophilic characteristics. Blood compatible materials are particularly preferred. Such preferred materials are biological materials including proteins such as collagen, gelatin or serum albumins or globulins, either derived from humans or having a structure similar to the human protein, glycosaminoglycans such

25 as hyaluronic acid, heparin and chondroitin sulphate and combinations or derivatives thereof. Synthetic biodegradable polymers, such as polyethylene glycol, polyethylene oxide, polypropylene glycol and combinations or derivatives may also be used. The outer layer is typically amphiphilic, as well as having a chemistry which allows charge and chemical modification. The versatility of the surface allows for such

30 modifications as altering the charge of the outer shell, such as by selecting a type A gelatin having an isoelectric point above physiological pH, or by using a type B gelatin having an isoelectric point below physiological pH. The outer surfaces may

also be chemically modified to enhance biocompatibility, such as by PEGylation, succinylation or amidation, as well as being chemically binding to the surface targeting moiety for binding to selected tissues. The targeting moieties may be antibodies, cell receptors, lectins, selectins, integrins or chemical structures or analogues of the receptor targets of such materials. The mechanical properties of the outer layer may also be modified, such as by cross linking, to make the microparticles suitable for passage to the left ventricle, to provide a particular resonant frequency for a selected harmonic of the diagnostic imaging system, or to provide stability to a threshold diagnostic imaging level of the ultrasound radiation.

10 The inner shell will be a biodegradable polymer, which may be a synthetic polymer. An advantage of the inner shell is that it provides additional mechanical or drug delivery properties to the microparticle which are not provided or insufficiently provided by the outer layer, or enhances mechanical properties not sufficiently provided by the outer layer, without being constrained by surface property requirements. For example, a biocompatible outer layer of a cross-linked proteinaceous hydrogel can be physically supported using a high modulus synthetic polymer as the inner layer. The polymer may be selected for its modulus of elasticity and elongation, which define the desired mechanical properties. Typical biodegradable polymers include polycaprolactone, polylactic acid, polylactic-polyglycolic acid co-polymers, co-polymers of lactides and lactones, such as epsilon-caprolactone, delta-valerolactone, polyalkylcyanoacrylates, polyamides, polyhydroxybutyrylates, polydioxanones, poly-beta-aminoketones, polyanhydrides, poly-(ortho)esters, polyamino acids, such as polyglutamic and polyaspartic acids or esters of polyglutamic and polyaspartic acids. References on many biodegradable polymers are cited in Langer, et. al. (1983) *Macromol.Chem.Phys.* C23, 61-125.

20 The inner layer permits the modification of the mechanical properties of the shell of the microparticle which are not provided by the outer layer alone. Moreover, the inner layer may provide a drug carrier and/or drug delivery capacity which is not sufficient or providable by the outer layer alone. For use as an ultrasonic contrast agent, the inner layer will typically have thickness which is no larger than is necessary to meet the minimum mechanical or drug carrying/delivering properties, in order to maximize the interior gas volume of the microparticle. The greater the gas

volume within the microparticle the better the echogenic properties.

Since the multilayer nature of the microparticle allows the ultrasound response to be tunable based on the choice of materials and combinations of materials in the different layers, the microparticles may be made so that they are detectable by
5 ultrasound without rupturing. This allows a microparticle sample to be imaged under one set of conditions, then, by changing the ultrasound conditions so that a threshold is reached where they rupture, the drug can be selectively released when the microparticles are in the vicinity of the heart.

The combined thickness of the outer and inner layers of the microparticle shell
10 will depend in part on the mechanical and drug carrying/delivering properties required of the microparticle, but typically the total shell thickness will be in the range of 25 to 750 nm.

The microparticles may be prepared by an emulsification process to control the sequential interfacial deposition of shell materials. Due to the amphiphilicity of
15 the material forming the outer layer, stable oil/water emulsions may be prepared having an inner phase to outer phase ratio approaching 3:1, without phase inversion, which can be dispersible in water to form stable organic phase droplets without the need for surfactants, viscosity enhancers or high shear rates.

Two solutions are prepared, the first being an aqueous solution of the outer
20 biomaterial. The second is a solution of the polymer which is used to form the inner layer, in a relatively volatile water-immiscible liquid which is a solvent for the polymer, and a relatively non-volatile water-immiscible liquid which is a non-solvent for the polymer. The relatively volatile water-immiscible solvent is typically a C5-C7 ester, such as isopropyl acetate. The relatively non-volatile water-immiscible non-
25 solvent is typically a C6-C20 hydrocarbon such as decane, undecane, cyclohexane, cyclooctane and the like. In the second solution containing the polymer for the inner layer, the polymer in water-immiscible solvents are combined so that the polymer fully dissolves and the two solvents are miscible with agitation. The polymer solution (organic phase) is slowly added to the biomaterial solution (aqueous phase) to form a
30 liquid foam. Typically about three parts of the organic polymer solution having a concentration of about 0.5 to 10 percent of the polymer is added to one part of the aqueous biomaterial solution having a concentration of about 1 to 20 percent of the

biomaterial. The relative concentrations of the solutions and the ratio of organic phase to aqueous phase utilized in this step essentially determine the size of the final microparticle and wall thickness. After thorough mixing of the liquid foam, it is dispersed into water and typically warmed to about 30 - 35°C with mild agitation.

5 While not intending to be bound by a particular theory, it is believed that the biomaterial in the foam disperses into the warm water to stabilize an emulsion of the polymer in the organic phase encapsulated within a biomaterial envelope. To render the biomaterial envelope water insoluble, a cross linking agent, such as glutaraldehyde, is added to the mixture to react with the biomaterial envelope and
10 render it water insoluble, stabilizing the outer shell. Other cross-linking agents may be used, including the use of carbodiimide cross-linkers.

Since at this point the inner core contains a solution of a polymer, a solvent and a non-solvent with different volatilities, as the more volatile solvent evaporates, or is diluted, the polymer precipitates in the presence of the less volatile non-solvent.
15 This process forms a film of precipitate at the interface with the inner surface of the biomaterial shell, thus forming the inner shell of the microparticle after the more volatile solvent has been reduced in concentration either by dilution, evaporation or the like. The core of the microparticle then contains predominately the organic non-solvent. The microparticles may then be isolated by centrifugation, washed,
20 formulated in a buffer system, if desired, and dried. Typically, drying by lyophilization removes not only the non-solvent liquid core but also the residual water to yield gas-filled hollow microparticles.

It may be desirable to further modify the surface of the microparticle, for example, in order to passivate surfaces against macrophages or the reticuloendothelial system (RES) in the liver. This may be accomplished, for example by chemically
25 modifying the surface of the microparticle to be negatively charged since negatively charged particles appear to better evade recognition by macrophages and the RES than positively charged particles. Also, the hydrophilicity of the surface may be changed by attaching hydrophilic conjugates, such as polyethylene glycol
30 (PEGylation) or succinic acid (succinylation) to the surface, either alone or in conjunction with the charge modification.

The biomaterial surface may also be modified to provide targeting

characteristics for the microparticle. The surface may be tagged by known methods with antibodies or biological receptors. The drug is released at the target site, for example, by increasing the ultrasonic energy to rupture the particles at the appropriate time and location.

5 The microparticles may also be sized or processed after manufacture. This is an advantage over lipid-like microparticles which may not be subjected to mechanical processing after they are formed due to their fragility.

 The final formulation of the microparticles after preparation, but prior to use, is in the form of a lyophilized cake. The later reconstitution of the microparticles may
10 be facilitated by lyophilization with bulking agents which provide a cake having a high porosity and surface area. The bulking agents may also increase the drying rate during lyophilization by providing channels for the water and solvent vapor to be removed. This also provides a higher surface area which would assist in the later reconstitution. Typical bulking agents are sugars such as dextrose, mannitol, sorbitol
15 and sucrose, and polymers such as PEG's and PVP's.

 It is undesirable for the microparticles to aggregate, either during formulation or during later reconstitution of the lyophilized material. Aggregation may be minimized by maintaining a pH of at least one to two pH units above or below the isoelectric point(P_i) of the biomaterial forming the outer surface. The charge on the
20 surface is determined by the pH of the formulation medium. Thus, for example, if the surface of the biomaterial has a P_i of 7 and the pH of the formulation medium is below 7, the microparticle will possess a net positive surface charge. Alternatively, if the pH of the formulation medium is greater than 7, the microparticle would possess a negative charge. The maximum potential for aggregation exist when the pH of the
25 formulation medium approaches the P_i of the biomaterial used in the outer shell. Therefore by maintaining a pH of the formulation medium at least one to two units above or below the P_i of the surface, microparticle aggregation will be minimized. As an alternative, the microparticles may be formulated at or near the P_i with the use of surfactants to stabilize against aggregation. In any event, buffer systems of the final
30 formulation to be injected into the subject should be physiologically compatible.

 The bulking agents utilized during lyophilization of the microparticles may also be used to control the osmolality of the final formulation for injection. An

osmolality other than physiological osmolality may be desirable during the lyophilization to minimize aggregation. However, when formulating the microparticles for use, the volume of liquid used to reconstitute the microparticles must take this into account.

5 Other additives may be included in order to prevent aggregation or to facilitate dispersion of the microparticles upon formulation. Surfactants may be used in the formulation such as poloxomers (polyethylene glycol-polypropylene glycol-polyethylene glycol block co-polymers). Water soluble polymers also may assist in the dispersion of the microparticles, such as medium molecular weight
10 polyethyleneglycols and low to medium molecular weight polyvinylpyrrolidones.

 The microparticles may be soaked in a solution of the drug whereby the solution diffuses into the interior. In particular, the use of bilayered microparticles where the inner shell has a porous characteristic allows for rapid diffusion of a drug solution into the hollow core. The microparticles may be re-dried such as by
15 lyophilization to produce a gas filled, drug containing microparticle. Alternatively, the drug may be dissolved in the organic phase with the biopolymer during the microparticle forming process. Evaporation of the organic solvents causes the drug to coprecipitate with the biopolymer inside the microparticle.

 It will be realized that various modifications of the above-described processes
20 may be provided without departing from the spirit and scope of the invention. For example, the wall thickness of both the outer and inner layers may be adjusted by varying the concentration of the components in the microparticle-forming solutions. The mechanical properties of the microparticles may be controlled, not only by the total wall thickness and thicknesses of the respective layers, but also by selection of
25 materials used in each of the layers by their modulus of elasticity and elongation, and degree of cross-linking of the layers. Upon certain conditions involving rapid deposition of the inner polymer or very low inner polymer content, porosity of the inner polymer shell is observed. The pores range from approximately 0.1 to 2 micron in diameter as observed under electron microscopy. Mechanical properties of the
30 layers may also be modified with plasticizers or other additives. Adjustment of the strength of the shell may be modified, for example, by the internal pressure within the microparticles. Precise acoustical characteristics of the microparticle may be

achieved by control of the shell mechanical properties, thickness, as well as narrow size distribution. The microparticles may be ruptured by ultrasonic energy to release gases trapped within the microparticles into the blood stream. In particular, by appropriately adjusting the mechanical properties, the particles may be made to remain stable to threshold diagnostic imaging power, while being rupturable by an increase in power and/or by being exposed to its resonant frequency. The resonant frequency can be made to be within the range of transmitted frequencies of diagnostic body imaging systems or can be a harmonic of such frequencies. During the formulation process the microparticles may be prepared to contain various gases, including blood soluble or blood insoluble gases. It is a feature of the invention that microparticle compositions may be made having a resonant frequency greater or equal to 2 MHz, and typically greater or equal to 5 MHz.

EXAMPLE 1

Controlled Rupture of Microcapsules **with Ultrasound In-Vitro**

A 6% aqueous solution was prepared from a 25% solution of USP grade human serum albumin (Alpha Therapeutic Corp) by dilution with deionized water. The solution was adjusted to a pH of 3.49 using 1 N HCl. Separately, 8 parts by weight polycaprolactone (M.W. 50,000) and 45 parts cyclooctane were dissolved in 300 parts isopropyl acetate at approximately 70 C. Once dissolution was complete, the organic solution was allowed to cool to 37 C. With mild stirring, 42.5 gm of the prepared organic solution was slowly incorporated into 25.0 gm of the albumin solution while the mixture was maintained at 30 C. The resulting coarse o-w emulsion was then circulated through a stainless steel sintered metal filter element having a nominal pore size of 7 microns. Recirculation of the emulsion was continued for 8 minutes. The emulsion was then added with stirring to 350 ml deionized water maintained at 30 C and containing 1.0 ml of 25% gluteraldehyde. During the addition, the pH of the bath was monitored to insure that it remained between 7 and 8. Final pH was 7.1. Low shear mixing was continued for approximately 2 ½ hours until the isopropyl acetate had completely volatilized. Poloxamer 188 in the amount of 0.75 gm was then dissolved into the bath. The

resulting microparticles were retrieved by centrifugation and washed 2 times in an aqueous solution of 0.25% poloxamer.

Microscopic inspection of the suspension revealed spherical particles having a thin-walled polymer shell with an outer protein layer and an organic liquid core. The peak diameter as, determined by the Malvern Micro particle size analyzer, was 4.12
5 microns.

The suspension was then lyophilized from a suspension in 25mM glycine, 0.5% Pluronic f-127, 0.1% sucrose, 3.0% mannitol and 5.0% PEG-3400. The resulting dry cake was reconstituted with deionized water and examined under the
10 microscope to reveal that the microparticles were spherical, discrete, and contained a gaseous core.

A test was performed to determine the ultrasound power threshold required to disrupt the capsule of this bilaminate microcapsule (POINT Biomedical biSphere™) made of albumin and polycaprolactone. The system was set up as follows. A water
15 tank 18" long by 12" wide by 4" deep was constructed using acrylic side walls and a plate glass bottom. A liquid flow loop was constructed to circulate the microsphere agent under test, consisting of a length of vinyl tubing, running through a peristaltic pump and connected to a section of thin walled silicone tubing to create a loop. The imaging was performed with a Hewlett-Packard Sonos 2500 ultrasound scanner. A
20 harmonic imaging transducer (1.8/3.6 MHz) was connected to the Sonos 2500, and the transducer head mounted on a test stand such that only the transducer face was immersed in the test tank. The flow loop was filled with de-gassed de-ionized water and the transducer was positioned to image the silicone tube.

With the transducer properly aligned, the Sonos 2500 parameters were
25 adjusted to give a clear image of the flow tube. The peristaltic pump was set to the lowest speed setting. After the set-up was complete, the Sonos 2500 was then switched to harmonic imaging mode (1.8/3.6 MHz) to observe the harmonic image response of the flow system. Without any contrast agent in the system, the silicone flow loop was barely visible. A small amount of the microcapsule suspension was
30 injected into the flow loop. Under harmonic imaging mode, the contrast agent was immediately visible flowing through the silicone imaging tube.

The Sonos 2500 was then switched into Pulse Wave (PW) Doppler mode and

a region of interest for the PW scan was centered on the apex of the silicone flow tube. Under PW mode, the ultrasound scanner converts the Doppler signal into an aural signal within the range of human hearing, and displays a repeating time vs intensity scan for the received signal. With the contrast agent flowing through the imaging tube, the transmission power was varied and the results evaluated on the ultrasound scanner screen and by listening to the aural signal from the converted data. At transmission power below 70% of the maximum, the PW received signal is a steady baseline, and the aural signal is a steady flow sound overlaid by the repetitive sound of the peristaltic flow from the pump. A sample of the flowing liquid was withdrawn from the injection port and visualized under a standard light microscope. The microcapsules were still intact.

The transmission power was then increased above 70% and the signal scan showed increasing high frequency spikes indicating the disruption of the capsules of the agent under test. The quantity of capsule disruption increased steadily with increasing power, up to the maximum output. The aural signal was a series of “click” sounds which also increased with increasing power. Again, a sample of the flowing liquid was withdrawn from the loop and checked with a light microscope. After ultrasound power input greater than 70%, the majority of the microsphere capsules appeared to be disrupted in some manner, usually fractured along an spherical plane, but in some cases reduced to wall fragments.

The results of the experiment were repeatable, even with the same sample flowing in the loop, i.e. decreasing the power below the 70% threshold stopped the capsule disruption and increasing back above the 70% threshold began disruption, a process that could be repeated many times until all of the agent capsules under test had been disrupted.

EXAMPLE 2

Controlled Rupture of Microcapsules with Ultrasound In-Vivo

A test similar to Example 1 was performed within the heart of a canine subject. Suspensions of a bilaminate albumin/polycaprolactone microsphere (POINT Biomedical biSphere™) were injected into an anesthetized dog and the wash-in of the

contrast effect observed in the chambers of the heart with a Hewlett Packard Sonos 2500 medical ultrasound imaging system, using a 1.8/3.6 MHz harmonic transducer placed over the heart. After several minutes, an apparent steady state of contrast brightness was achieved in the heart. The ultrasound system was set to Pulse Wave
5 (PW) Doppler mode as performed in Example 1, and the heart imaged. With the region of interest set within the left ventricle, an increase in ultrasound output power created a series of audible clicks and corresponding sharp peaks on the graphic Doppler display of the ultrasound system, indicating the rupturing of the microcapsules as demonstrated in-vitro. Moving the region of interest to the
10 myocardium still produced audible clicks and graphic peaks, but a reduced level, indicating microcapsule rupture within the reduced blood volume of the myocardium.

EXAMPLE 3

Dye Loading Of Albumin Polylactide Microparticles

15 A 6% aqueous solution was prepared from a 25% solution of USP grade human albumin by dilution with deionized water. Ion exchange resin (AG 501-X8, BioRad Laboratories) was then added to the solution at a ratio of 1.5 gm resin to 1.0 gm dry weight of albumin. After 3 hours the resin was removed by filtration and the pH of the solution was adjusted from 4.65 to 5.5 Separately, 0.41 gm d-l lactide (0.69
20 dL/gm in CHCl₃: at 30 C) and 5.63 gm cyclooctane were dissolved in 37.5 gm isopropyl acetate. The organic solution was then slowly incorporated into 25.0 gm of the prepared albumin solution with mild stirring while the mixture was maintained at 30 C. The resulting coarse o-w emulsion was then circulated through a stainless steel sintered metal filter element having a nominal pore size of 7 microns. Recirculation of
25 the emulsion was continued for 8 minutes. The emulsion was then added with stirring to 350 ml deionized water maintained at 30 C and containing 1.0 ml of 25% gluteraldehyde. During the addition, the pH of the bath was monitored to insure that it remained between 7 and 8. Final pH was 7.0. Low shear mixing was continued for approximately 2½ hours until the isopropyl acetate had completely volatilized.
30 Polyoxamer 188 in the amount of 0.75 gm was then dissolved into the bath. The resulting microspheres were retrieved by centrifugation and washed 2 times in an aqueous solution of 0.25% polyoxamer.

Microscopic inspection revealed hollow spherical polymer microparticles having an outer protein layer and an inner organic liquid core. The suspension was formulated with a glycine/PEG 3350 excipient solution, then lyophilized. The resulting dry cake was reconstituted with deionized water and examined under the microscope to reveal that the microparticles were spherical, discrete, and contained a gaseous core.

A lyophilized cake in a 10 ml serum vial, composed of excipient and the lactide-containing microparticles was placed into a 50 ml centrifuge tube. Enough isopropyl alcohol was added to cover the cake and it was allowed to soak for 30 seconds. Aqueous Pluronic F68 solution (0.25% w/w) was added to fill the tube. After centrifuging, the supernatant was removed and another rinse performed. A saturated, filtered solution of rhodamine B was added to the microparticles and allowed to soak overnight. Under the microscope, the microparticles appeared filled with dye solution. A dye saturated F68 solution was made to use as a lyophilization excipient. Four ml of the excipient was combined with the approximately 2 ml of microcapsule containing solution and the resulting mixture was split between two 10 ml serum vials. The vials were frozen at -80°C and lyophilized in a FTS tray dryer. Both vials were purged with perfluorobutane gas by five pump-down purge cycles with a vacuum pump. Observation showed some microparticles that were half full of red solution and half full of gas. There was no obvious leakage of the dye from these microparticles during observation. The microparticles were rinsed with four, 20 ml portions of F68 solution on a vacuum filter. The microparticles were placed in a cuvette, centrifuged, and an initial spectra was taken. The cuvette was sonicated in an ultrasonic bath, centrifuged, and another spectra taken.

25

Abs. Initial (553-800)

Abs. Sonicated (553-800)

1.164

1.86

The higher absorption after sonication indicates that encapsulated dye was released upon insonation of the microparticles.

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EXAMPLE 4Dye loading of Human Serum AlbuminPolycaprolactone Microparticles

A lyophilized cake in a 10 ml serum vial, composed of excipient and paraffin-
5 containing microparticles prepared in accordance with example 1 was placed into a 50
ml centrifuge tube. The only modification was that 0.2 gm paraffin was added with
the polycaprolactone in cyclooctane. The cake was covered with methanol and
allowed to soak for 30 seconds. The tube was then filled with an aqueous solution of
0.25% (w/w) Pluronic F68, gently mixed, and centrifuged in order to precipitate the
10 now fluid-filled microcapsules. The supernatant was removed and the tubes were
again filled with pluronic solution. The microparticles were resuspended by
vortexing and again centrifuged. After removing the supernatant solution, two ml of a
saturated, filtered solution of brilliant blue G dye in 0.25% (w/w) aqueous F68 was
added. The microparticles were allowed to soak for approximately 72 hours.
15 Microscopic examination revealed 90-95% of the microparticles to be filled with dye
solution. A lyophilization excipient was prepared. Four ml of the excipient was
added to the microparticle solution and mixed by vortexing. Two 10 ml serum vials
were filled with 3 ml each of solution and frozen at -80°C. The vials were lyophilized
on a FTS flask lyophilizer. Both vials and a portion of deionized water were purged
20 with perfluorobutane for 10 minutes. Both vials were reconstituted with deionized
water and rinsed with two 40 ml portions of 0.25% (w/w) F68 solution on a vacuum
filter. The resulting microparticle solution was split into two 3 ml portions. One
portion was sonicated in an ultrasonic bath to rupture the bubbles. Both portions were
diluted 1/10 with F68 solution and placed into UV-visible cuvettes. The cuvettes
25 were centrifuged and a visible spectra was taken.

Absorption (at 605nm-800nm)

Sonicated	0.193
Non-sonicated	0.136

30 The higher absorption after sonication indicates that encapsulated dye was
released upon insonation of the microcapsules.

WHAT IS CLAIMED IS:

1. A microparticle composition containing biodegradable microparticles having diameters within the range of about 1 to 10 microns, wherein said
5 microparticles have hollow cores containing a gas and at least one drug, wherein said microparticles are rupturable with ultrasound at a pre-determined ultrasound energy condition to release a drug within the heart or within tissues of the heart.
2. A microparticle composition of claim 1 where the drug comprises an
10 agent to limit ischemic injury to the heart.
3. A microparticle composition of claim 1 where the drug comprises an agent to limit reperfusion injury to the heart.
- 15 4. A microparticle composition of claim 1 where the drug comprises an agent to limit restenosis of a coronary artery.
5. A microparticle composition of claim 1 where the drug comprises a
20 fibrinolytic agent, vasodilator, calcium channel blocker, angiogenesis agent, anti-platelet agent, anti-white cell agent, endocardium acting agent, free radical scavenging agent, or anti-restenosis agent.
6. A microparticle composition of claim 2 where the drug comprises
25 adenosine, adenosine monophosphate, adenosine diphosphate, adenosine triphosphate or chemical derivatives of adenosine.
7. The microparticle composition of claim 1 where the microparticles
comprise biodegradable polymers.
- 30 8. The microparticle composition of claim 1 where the microparticles are imageable with ultrasound under a first set of conditions without rupturing the microparticles, then rupturable by ultrasound under a second set of conditions which

is a characteristic rupture threshold of the microparticles at which the microparticles rupture and release drugs.

9. The microparticle composition of claim 1 where the circulatory half-
5 life of the drug incorporated into said microparticle composition is greater than the circulatory half-life of the drug administered alone.

10. The microparticle composition of claim 5 where the drug comprises a
10 vasodilator.

11. The microparticle composition of claim 10 where the drug is released
within the heart by ultrasound to assess flow reserve of the heart.

12. A microparticle composition of claim 2, 3 or 5 wherein said
15 microparticles contain a plurality drugs.

13. A method of delivering a drug to the heart of a subject comprising the
steps of:

a) introducing microparticles incorporating a pharmaceutical
20 agent into the bloodstream of said subject, said microparticles being rupturable at a pre-determined ultrasound condition;

b) monitoring the location of said microparticles within said
subject to determine presence of said microparticles within tissues of the heart;

c) directing ultrasound energy at said tissues sufficient to achieve
25 said ultrasound condition to induce release of said pharmacological agent in the heart from said microparticles to achieve a pharmacological effect.

14. The method of claim 13 wherein said pharmacologic agent
comprises a fibrinolytic agent, vasodilator, calcium channel blocker, angiogenesis
30 agent, anti-platelet agent, anti-white cell agent, endocardium acting agent, free radical scavenging agent, or anti-restenosis agent.

15. The method of claim 13 wherein the circulatory half-life of said agent incorporated into said microparticles is increased over the circulatory half-life of the drug administered alone.
- 5 16. The method of claim 13 wherein the systemic toxicity of said agent is reduced when incorporated into said microparticles.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20388

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(7) :Please See Extra Sheet.
 US CL :424/9.1, 9.2, 9.32, 9.5, 400, 408, 425, 450, 451
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
 U.S. : 424/9.1, 9.2, 9.32, 9.5, 400, 408, 425, 450, 451

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,650,156 A (GRINSTAFF et al.) 22 July 1997, col. 8, lines 1-35, col. 10, lines 1-45, col. 20, lines 35-62, col. 39, lines 20-55.	1, 2, 7, 8, 11-13
Y,P	US 5,858,990 A (WALSH) 12 January 1999, col. 8, lines 1-65, col. 9, lines 34-55, col. 17, lines 50-67, col. 18, lines 1-60.	1-16
X	US 4,962,091 A (EPPSTEIN et al.) 09 October 1990, col. 13, lines 30-67, col. 21, lines 55-67.	1, 3, 5-8, 10-14

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*&* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search: 16 DECEMBER 1999
 Date of mailing of the international search report: 10 FEB 2000

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/20388

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

A61K 9/00, 9/48, 9/129, 49/00; A61B 5/055, 8/00; A61F 2/00; A01N 25/34