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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 April 2002 (25.04.2002)

PCT

(10) International Publication Number
WO 02/32395 A2

(51) International Patent Classification[7]: A61K 9/00

(21) International Application Number: PCT/IL01/00966

(22) International Filing Date: 18 October 2001 (18.10.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
139177 20 October 2000 (20.10.2000) IL

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

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

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

WO 02/32395 A2

(54) Title: SUSTAINED RELEASE DELIVERY SYSTEM AND USES THEREOF

(57) Abstract: The invention relates to a sustained release delivery system for the delivery of an active agent to a warm-blooded animal and to uses thereof. The delivery system comprises an aqueous bicellar matrix that is liquid at temperatures below ambient temperature and forms a biodegradable gel at body temperature of said animal and an active agent, and optionally further comprises pharmaceutically acceptable additive, carrier and/or diluent. The aqueous bicellar matrix is preferably a mixture of a lipid, preferably phospholipid, and a detergent in water.

Sustained Release Delivery System and Uses Thereof

Field of the Invention

This invention relates to the field of sustained release systems for delivery of desired agents to warm-blooded animals, and particularly to the use of thermally-gelling lipid-detergent mixtures as matrices of delivery systems, for facilitated administration of active agents such as bio-active, radio- and chemo-therapeutic substances. The invention is based on an aqueous mixture of phospholipids of different lengths and concentrations, that is a real solution at ambient or lower temperatures, but forms a semi-solid or gel at warmer, particularly body temperature. When exposed to aqueous environments the gel decomposes gradually, releasing the compounds entrapped therein.

Background of the Invention

Drug delivery systems

In many cases a high molecular weight of a drug, degradation in the gastrointestinal tract and short half-life in the body, require parenteral administration. In many cases, repeated administrations are required in order to achieve the expected therapeutic effect for an extended period of time. Long-term sustained delivery of such drugs is essential for the practical use of these medications. Another problem associated with frequent administration is patient compliance. It is often difficult to get a patient to follow a prescribed dosage regimen, particularly when the prescription is for a chronic disorder and the drug has side effects. Therefore, it would be highly desirable to provide a system for the delivery of drugs at a sustained rate over a long period of time, free of those problems, in order to optimize the therapeutic efficacy, minimize side-effects and toxicity, and enhance patient compliance.

Drug-loaded polymeric devices have been tried for long term treatment of various diseases. An important requirement of such a polymer is bio- degradability, meaning that the polymer can break down, or be degraded within the body into nontoxic components. This may take place either concomitantly with the release of the drug, or after completion of the release. Furthermore, the techniques, procedures, solvents and other additives used to make the device and load the drug should result in dosage forms that are safe for the patient, minimize irritation to the surrounding tissue, and constitute a compatible medium for the drug.

Biodegradable implantable controlled release devices are currently made of solid polymers such as polyglycolic acid (PLGA), polylactic acid (PLA), or copolymers of glycolic and lactic acid. In most cases, the final polymeric device is fabricated in a distinct solid shape (e.g., sphere, slab or rod) requiring implantation that is often accompanied by the trauma of a surgical procedure.

Although there has been some limited success, such polymers have problems inherent to their physicochemical properties and methods of fabrication.

Transfection techniques

Transfection, i.e. the introduction into a living eukaryotic cell of DNA or mRNA (messenger RNA) capable of being expressed in the cell, constitutes an alternative approach to the intracellular introduction of polypeptides and proteins. This technique finds applications in numerous fields, ranging from cell biology to medicine. In cell biology, transfection techniques can be used especially to study the intracellular role of cloned gene products and to study the regulation of gene expression. Transfection is also used in gene therapy for the

correction of genetic disorders; it also finds application in the field of therapeutic peptides and in immunization with the development of polynucleotide vaccines.

There are known in the prior art numerous transfection methods, in particular precipitation of DNA and calcium phosphate or DEAE-dextran, or alternatively, the use of cationic lipids in the form of liposomes which form complexes with negatively charged polynucleotides and facilitate their transmembrane passage.

Although a number of these products are commercially available and are effective for the transfection of cells in culture, their efficacy *in vivo* is limited.

Radiotherapy

Radiation has been used for cancer therapy and for the control of local healing in areas as diverse as the prevention of excessive scar formation or reduction of lymphoid infiltration and proliferation. More recently, radiation has been used to inhibit restenosis following coronary artery or peripheral artery angioplasty.

The delivery of radiation by an extracorporeal beam is encountered with the usual problems of limiting the exposure to only those tissues intended to be affected. Moreover, doses must often be subdivided, requiring repeated visits to the clinic.

An alternative approach involves the use of immobilized radionuclides. In this approach, depots are used as a vehicle for the immobilization and local delivery of a radionuclide or a radiopharmaceutical. Radionuclides are incorporated in their elemental forms (as inorganic compounds), or are attached to a larger molecule or incorporated into

the delivery system by physical or chemical methods. The depot is preferably made of a biodegradable material designed to degrade at a known rate under conditions encountered at the site of application.

The depot is preferably fluent, or capable of being made fluent, so that it may be deposited at a site with minimal invasion. The use of polymeric depots provides means of immobilizing the source of radiation at a remote site within the body, which can be accessible by a less invasive surgical procedure (catheter or laparoscope).

The duration and total dose of radiation can be controlled by a combination of the choice of the radionuclide, control of the rate of degradation of the polymer, and control of the rate of release of the radionuclide from the depot.

It is thus an object of the present invention, to provide an easily administrable, bio- and drug-compatible sustained release system for the delivery of therapeutic agents to warm-blooded animals, that will overcome the drawbacks of known delivery devices.

It is a further object of the invention to provide an efficient transfection system for transfection of cells with nucleic acids.

Yet another object of the invention is to provide a radiotherapeutic delivery system.

These and other objects of the invention will become more apparent as the description proceeds.

Summary of the Invention

The invention relates to a sustained release delivery system for the delivery of an active agent to a warm-blooded animal, the system comprising an aqueous bicellar matrix that is liquid at temperatures below ambient temperature and forms a biodegradable gel at body temperature of said animal and an active agent, and optionally further comprising pharmaceutically acceptable additive, carrier and/or diluent.

More particularly, the invention relates to a sustained release delivery system for the delivery of an active agent to a warm blooded animal comprising an aqueous bicellar matrix that is liquid at temperatures below ambient temperature and forms a biodegradable gel at body temperature of said animal, an active agent; and an additive being a pH adjusting agent, and optionally further comprising pharmaceutically acceptable carrier and/or diluent.

In preferred embodiments, the matrix comprises a mixture of a lipid, preferably phospholipid, and a detergent in water. The lipid is preferably a long chain phosphatidylcholine, and more particularly a long chain phosphatidylcholine selected from the group consisting of dimyristoylphosphatidylcholine (DMPC) and hydrolysis-resistant dialkyl analogs thereof such as 1,2-O-ditetradecyl-sn-glycero-phosphocholine or 1,2-O-di-dodecyl-sn-glycero-phosphocholine. A particularly preferred long chain phosphatidylcholine is DMPC.

A particular detergent may be dihexanoylphosphatidylcholine (DHPC) or analogs thereof such as 1,2-O-dihexyl-*sn*-glycero-phosphocholine.

Sustained release delivery systems according to the invention may have a total lipid concentration in the range of about 3% to 40% w/v, for example from 15% to 35%, particularly from 20% to 30%, and the molar ratio between DMPC and DHPC is in the range of from 4:1 to 1.5:1, for example from 3:1 to 2:1, and particularly from 2.9:1 to 2.4:1.

In another specific embodiment, the detergent may be 3-(cholamidopropyl)- dimethylammonio- 2-hydroxy-1-propane sulfonate (CHAPSO).

Sustained release delivery systems of the invention may have a total lipid concentration in the range of about 5% to 40% w/v, and the molar ratio between DMPC and CHAPSO in the range of from 5:1 to 2:1.

Another specific detergent is glycocholate.

Sustained release delivery systems of the invention may have a total lipid concentration in the range of about 45% to 65% w/v, and the molar ratio between DMPC and glycocholate is in the range of from 2:1 to 8:1.

The sustained release delivery system of the invention may further comprise positively or negatively charged phospholipids. The concentration of said charged phospholipids may be about 0.01%-5% w/v.

The sustained release delivery system of the invention may contain as active agent at least one peptide or protein, preferably selected from the group consisting of oxytocin, vasopressin, adrenocorticotropic

hormone, epidermal growth factor, platelet-derived growth factor (PDGF), prolactin, luliberin ,luteinizing hormone releasing hormone (LHRH), LHRH agonists, LHRH antagonists, growth hormone (human, porcine, bovine, etc.), growth hormone releasing factor, insulin, somatostatin, glucagon, interleukin-2 (IL-2), interferon-alpha, -beta, or -gamma, gastrin, tetragastrin, pentagastrin, urogastrone, secretin, calcitonin, enkephalins, endorphins, angiotensins, thyrotropin releasing hormone (TRH), tumor necrosis factor (TNF), nerve growth factor (NGF), granulocyte-colony-stimulating factor (G-CSF) granulocyte-macrophage-colony-stimulating factor (GM-CSF), macrophage- colony-stimulating factor (M-CSF), heparinase, bone morphogenic protein (BMP), hANP, glucagon-like peptide (GLP-1), interleukin- 11 (IL-11), renin, bradykinin, bacitracins, polymyxins, colistins, tyrocidine, gramicidins, cyclosporins, enzymes, cytokines, antibodies and monoclonal antibodies and pharmaceutically active synthetic analogues, derivatives, modifications and fragments thereof.

Alternatively, the active agent may be at least one anticancer agent, preferably selected from the group consisting of mitomycin, bleomycin, BCNU, carboplatin, doxorubicin, daunorubicin, methotrexate, paclitaxel, taxotere, actinomycin D, and camptothecin.

Still alternatively, the sustained release delivery system of the invention may contain as said active agent at least one antibacterial substance, preferably selected from the group consisting of beta-lactam antibiotics, tetracyclines, chloramphenicol, neomycin, gramicidin, bacitracin, sulfonamides, aminoglycoside antibiotics, tobramycin, nitrofurazone, nalidixic acid and analogs, the antimicrobial combination of fludalanine/pentizidone, nitrofurazone, cephalosporins, chlortetracyclin, clindamycin, erythromycins, gentamicin, ofloxacin, penicillins and streptomycin.

The active agent may also be at least one bacteriostatic agent, preferably selected from the group consisting of iodine, chloramines, benzalkonium chloride and phenol.

Further, the said active agent may be at least one steroidal or non-steroidal anti-inflammatory drug, preferably selected from the group consisting of cortisone, hydrocortisone, betamethasone, dexamethasone, fluocortolone, prednisolone, triamcinolone, indomethacin, sulindac and its salts and corresponding sulfide.

The active agent may further be at least one antiparasitic agent, preferably selected from the group consisting of ivermectin, tetrahydropyrimidine, benzimidazol, quinoline and molevac.

Alternatively, the sustained release delivery system of the invention may contain as said active agent at least one antiviral agent, preferably selected from the group consisting of acyclovir, interferon, amantadine, famciclovir, valacyclovir, oseltamivir, ganciclovir, idoxuridine, vidarabine, trifluridine.

The sustained release delivery system of the invention may also contain as said active agent at least one anesthetic agent, preferably selected from the group consisting of benzocaine, lidocaine and dibucaine.

In addition, the sustained release delivery system of the invention may contain as said active agent an antifungal agent, preferably selected from the group consisting of tolnaftate, undecylenic acid, salicylic acid, zinc undecylenate and thiabendazole, amphotericin, butenafine, ciclopiroxolamine, clotrimazole, econazole, flucomazol, fungilin,

galivert, heralvent, oricant, grapefruit seed extract, hylac forte, ketoconazole, miconazole, mucokehl, mutaflor, paidoflor, naftifine, natamycin, nystatin, omnifloran, pefrakehl, prosymbioflor, symbioflor 1 and 2, taheebo, trenev-trio.

Still further, the sustained release delivery system of the invention may contain as said active agent is an analgesic agent, preferably selected from the group consisting of methylsalicylate, menthol, camphor, methylnicotinate, triethanolamine salicylate, glycol salicylate and salicylamine, salsalate, phenacetin, p-aminosalicyl acid, diflunisal, salicylate combinations, tramadol, propoxyphene, morphine sulfate, hydromorphone, meperidine, pentazocine and pentazocine combinations, and the combinations: codeine phosphate/acetaminophen, propoxyphene HCl/aspirin, propoxyphene HCl/acetaminophen, hydrocodone bitartrate/aspirin, hydrocodone bitartrate/ acetaminophen, hydrocodone/ ibuprofen, oxycodone/acetaminophen and oxycodone/aspirin.

Other active agents may be smoking depressants and drugs for male and female sexual disorders.

In yet a further embodiment the sustained release delivery system of the invention comprises as said active agent a radionuclide, preferably selected from the group consisting of iodine, iridium, radium, cesium and yttrium.

In another embodiment, the sustained release delivery system of the invention comprises as said active agent is a depilatory agent, such as sodium or calcium thioglycolate.

Still further, the sustained release delivery system of the invention may contain as said active agent at least one transfecting agent, preferably selected from the group consisting of plasmids and/or polynucleotides.

All of the sustained release delivery systems of the invention may optionally further contain pharmaceutically acceptable additives, carriers and/or diluents.

In another aspect, the invention relates to a radiotherapeutic composition for providing radioactive therapy to a warm-blooded animal, comprising a radioactive aqueous bicellar matrix that is liquid at temperatures below ambient temperature and forms a biodegradable gel at body temperature (of said animal) and optionally further comprising pharmaceutically acceptable additive, carrier and/or diluent.

The radiotherapeutic composition of the invention may comprise as an additive a pH adjusting agent, and may optionally further comprise pharmaceutically acceptable carrier and/or diluent.

In the radiotherapeutic composition of the invention, said matrix preferably comprises a mixture of a lipid, preferably phospholipid, and a detergent in water, wherein all or part of any of said lipid or detergent is radioactively labeled.

Also in this aspect, the said lipid is preferably a long chain phosphatidylcholine, more particularly a long chain phosphatidylcholine selected from the group consisting of dimyristoylphosphatidylcholine (DMPC) and hydrolysis-resistant dialkyl analogs thereof such as 1,2-O-ditetradecyl-sn-glycero-

phosphocholine or 1,2-O-di-dodecyl-sn-glycero-phosphocholine. A particularly preferred long chain phosphatidylcholine is DMPC.

A particular detergent may be dihexanoylphosphatidylcholine (DHPC) and analogs thereof such as 1,2-O-dihexyl-*sn*-glycero-phosphocholine.

Sustained release delivery systems according to the invention may have a total lipid concentration in the range of about 3% to 40% w/v, for example from 15% to 35%, particularly from 20% to 30%, and the molar ratio between DMPC and DHPC is in the range of from 4:1 to 1.5:1, for example from 3:1 to 2:1, particularly from 2.9:1 to 2.4:1.

In another specific embodiment, the detergent may be 3-(cholamidopropyl)- dimethylammonio- 2-hydroxy-1-propane sulfonate (CHAPSO).

Sustained release delivery systems of the invention may have a total lipid concentration in the range of about 5% to 40% w/v,–and the molar ratio between DMPC and CHAPSO in the range of from 5:1 to 2:1.

Another specific detergent is glycocholate.

Sustained release delivery systems of the invention may have a total lipid concentration in the range of about 45% to 65% w/v, and the molar ratio between DMPC and glycocholate is in the range of from 2:1 to 8:1.

In a further embodiment, the delivery system of the invention may contain as the active agent a vaccinating agent, in admixture with an immunogenic agent that elicits an immune response. The

immunogenic agent may be selected from the group consisting of killed bacteria, preferably killed mycobacteria. The vaccinating agent may be any available vaccinating agent, e.g. vaccines against hepatitis, rubella, polio, influenza, tuberculosis etc.

Still further, the invention relates to a method of treating a medical condition in a patient in need of such treatment, comprising parenterally administering to said patient a pharmaceutical composition comprising a bicellar matrix that is liquid at ambient temperature and forms a biodegradable gel upon administration to said patient, a therapeutically effective amount of a drug and a pH adjusting agent, said composition further optionally comprising a physiologically acceptable carrier and/or diluent.

Also when used in the method of the invention, the said matrix comprises an aqueous mixture of a lipid, preferably phospholipid, and a detergent. The phospholipid is preferably a long chain phosphatidylcholine.

The invention also relates to a method of providing radiotherapy to a patient in need of such treatment, comprising parenterally administering to said patient a radiotherapeutic composition comprising an aqueous mixture of a lipid and a detergent, which mixture is liquid at ambient temperature and forms a biodegradable gel upon administration to said patient, a therapeutically effective amount of radionuclide and a pH adjusting agent, said composition further optionally comprising a physiologically acceptable carrier and/or diluent.

Also in the radiotherapeutic method of the invention, the said matrix preferably comprises an aqueous mixture of a lipid, preferably

phospholipid, and a detergent. The phospholipid is preferably a long chain phosphatidylcholine.

In another aspect, the invention relates to a method of providing radiotherapy to a patient in need of such treatment, comprising parenterally administering to said patient a radiotherapeutic composition comprising an aqueous mixture of a lipid, preferably phospholipid, with a detergent, which mixture is liquid at ambient temperature and forms a biodegradable gel upon administration to said patient, a pH adjusting agent, wherein at least one of said lipid and detergent is radioactively labeled, said composition further optionally comprising a physiologically acceptable carrier and/or diluent. Also here, the matrix comprises an aqueous mixture of a lipid, preferably phospholipid, and a detergent. The lipid is preferably a long chain phosphatidylcholine.

Still further, the invention relates to method for providing efficient vaccination to a patient in need thereof, comprising parenterally administering to said patient a pharmaceutical composition comprising a bicellar matrix that is liquid at ambient temperature and forms a biodegradable gel upon administration to said patient, an effective amount of an immunogenic agent that elicits an immune response, the vaccinating agent and a pH adjusting agent, said composition further optionally comprising a physiologically acceptable carrier and/or diluent. Also here, the said matrix comprises an aqueous mixture of a lipid, preferably phospholipid, and a detergent. The said lipid is preferably a long chain phosphatidylcholine.

The invention further relates to a method of transfecting cells comprising the steps of: providing cells to be transfected; providing a bicellar matrix that is liquid at ambient temperature and forms a

biodegradable gel at a temperature above 15°C, said matrix containing a transfecting agent, a pH adjusting agent and optionally physiologically acceptable carrier and/or diluent; and contacting said cells with the bicellar matrix provided in step (b) and maintaining the mixture at a temperature above 15°C. The temperature is preferably above 25°C, particularly above 30°C and preferably about 37°C.

For the transfection method of the invention, the bicellar matrix comprises an aqueous mixture of a lipid, preferably phospholipid, and a detergent. The lipid is preferably a long chain phosphatidylcholine.

The transfecting agent may be an oligonucleotide, a polynucleotide, a gene and any DNA vector containing the same. Oligonucleotides can be antisense oligonucleotides. The said gene preferably encodes a therapeutic protein or protein product or is itself a therapeutic product.

Thus, the invention further relates to a method of *in vivo* transfecting cells in a patient in need of such treatment, comprising parenterally administering to said patient a bicellar matrix that is liquid at ambient temperature and forms a biodegradable gel at body temperature, said matrix containing a transfecting agent, a pH adjusting agent and optionally physiologically acceptable carrier and/or diluent. Also in this method, the said bicellar matrix comprises an aqueous mixture of a lipid, preferably phospholipid, and a detergent. The lipid is preferably a long chain phosphatidylcholine. The transfecting agent is any one of an oligonucleotide, a polynucleotide, a gene and any DNA vector containing the same. The said gene preferably encodes a therapeutic protein or protein product or is itself a therapeutic product.

Still further, the invention relates to a method of removing hair from the skin of a subject, comprising applying to the skin of said subject a pharmaceutical composition comprising a bicellar matrix that is liquid at ambient temperature and forms a biodegradable gel upon contact with the skin, a depilatory agent and a pH adjusting agent, said composition further optionally comprising a physiologically acceptable carrier and/or diluent. The said matrix comprises an aqueous mixture of a lipid, preferably phospholipid and a detergent. Said lipid is preferably a long chain phosphatidylcholine.

Brief Description of Figures

The invention will be described in more detail on hand of the following Figures in which:

Figure 1 shows the release profiles of toluidine blue from a gelled bicellar phase and from an aqueous solution. The square tracings depict the diffusion of toluidine blue from an aqueous solution placed inside a dialysis tubing (empty and filled squares represent two repetitions of the same experiment). The circle tracings represent the release profile of toluidine blue from a gelled bicellar phase placed in a dialysis tubing (empty and filled circles represent two repetitions of the same experiment).

Figure 2 shows a schematic diagram of a bicellar system in an external magnetic field. The bicelles are aligned with their director perpendicular to the magnetic field.

Figure 3 shows the components and general morphology of a disk shaped micelle (bicelle). The chemical structures of: (a) DMPC, (b) DHPC, (c) CHAPSO. (d) A model DHPC-DMPC bicelle. DMPC forms an

L_α type bilayer section surrounded by a rim of DHPC. (e) A model CHAPSO-DMPC bicelle depicted to contain an integral membrane protein.

Figure 4 depicts the effects of lipid to detergent ratio and water content on the system showing the gradual change from an extended lamelar phase through a magnetically oriented bicellar system to an isotropic solution.

Figure 5 depicts alkaline phosphatase expression in rat tibilalis muscle 3 days after a single IM injection of pDNA-bicell formulations. Mean ± SEM, n=6-8, $p < 0.05$.

Terms and Definitions

For the sake of clarity the meaning of the terms listed herein and as used herein are:

"Parenteral" shall mean by some means other than through the gastrointestinal tract or external-topical.

"T.m" or "gelation temperature" or "gel-sol transition temperature" mean the temperature at which the system undergoes reverse thermal gelation, i.e., the temperature below which the bicellar system is liquid and above which the bicellar system undergoes phase transition to increase in viscosity or to form a semi-solid gel.

The terms "gelation temperature" and "reverse thermal gelation temperature" or the like, shall be used interchangeably in referring to the gelation temperature.

"Reverse thermal gelation" is the phenomena whereby a solution of the bicellar system spontaneously increases in viscosity and transforms into a semisolid gel, as the temperature of the solution is increased above the gelation temperature of the bicellar system. When cooled below the gelation temperature, the gel spontaneously reverses to re-form the lower viscosity solution. This cycling between the solution and the gel may be repeated *ad infinitum*, because the sol/gel transition does not involve any change in the chemical composition of the system. All interactions to create the gel are physical in nature and do not involve the formation or breaking of covalent bonds.

"Drug delivery system having reverse thermal gelation properties" shall mean a bicellar solution that contains drugs or other compounds (the drug per se can be either dissolved or colloidal) suitable for

administration to a warm-blooded animal, which forms a gelled drug depot when the temperature rises to or above the gelation temperature of the system.

"Depot" means a drug delivery system following administration to a warm-blooded animal, which has formed a gel upon administration to the body, the temperature being raised to or above the gelation temperature.

"Gel" means the semi-solid phase that spontaneously occurs as the temperature of the "drug delivery system" is raised to or above the gelation temperature of the system.

"Bicellar system" or "Aqueous bicellar system" mean an aqueous liquid or gel-like mixture of lipids of different lengths or mixtures of lipids and detergents (and optionally additional biologically inert components). At temperatures below the gelation temperature the bicellar system will be a solution. At temperatures at or above the gelation temperature the bicellar system will solidify to form a gel. The terms "aqueous bicellar system", "bicellar system", "aqueous bicellar phase", "bicellar phase", "aqueous bicellar matrix" and "bicellar matrix" are used herein interchangeably.

"Aqueous bicellar composition" means either a drug delivery liquid or a gel comprised of the water phase having uniformly contained therein a drug or other agent and the lipid components of the bicellar system. At temperatures below the gelation temperature the bicellar system will be a solution. At temperatures at or above the gelation temperature the bicellar system will solidify to form a gel. In embodiments in which a lipid component of the bicellar system is also the active agent, the term means the liquid or gel comprised of the

water phase having uniformly contained therein the lipid components of the bicellar system.

"pH adjusting agent" means any physiologically acceptable acid, base or buffer capable of changing to and/or maintaining the acidity of the mixture to a physiologically acceptable value.

"Pharmaceutically effective amount" means the quantity of any substance required to effect the cure of a disease or that will correct the manifestation of a deficiency of a particular factor.

"Pharmaceutically acceptable or physiologically acceptable additive, carrier and/or diluent" mean any additive, carrier or diluent that is non therapeutic and non-toxic to recipients at the dosages and concentrations employed, and that does not affect the pharmacological or physiological activity of the active agent.

"Radiotherapeutically effective amount" means the quantity of radiation required to effect the cure of a disease or that will correct the manifestation of a deficiency of a particular factor.

"Long chain phosphatidylcholine" means a phosphatidylcholine with more than a total of 18 carbons in both chains.

"Total lipid concentration" means the weight per volume (w/v) content of detergent + phosphatidylcholine in the mixture.

Detailed Description of the Invention

There exists a need to provide a system for the delivery of active agents such as drugs and/or radiation at a sustained rate over a

prolonged period of time in order to optimize the therapeutic efficacy, minimize side effects and toxicity, and increase patient compliance. Such a system should be easily introduced without complex surgical intervention. In search for such system, the present inventor proposed that an adequate system should be liquid at room temperature, enabling easy mechanical manipulation such as introduction of the active principal and syringeability, and should gel at body temperatures, thus creating a depot containing the selected active agents. Such a system must meet the strict regulatory compliance requirements of biocompatibility, clearly defined pathway of degradation of the bicellar matrix components, and safety and lack of toxicity of the degradation products. It is also desirable that such a delivery system should be able to contain and release hydrophilic as well as hydrophobic active agents/compounds.

One system, which can be fabricated in aqueous solution, is based on a class of block copolymers, marketed under Pluronic^{R}. These copolymers are composed of two different polymer blocks, i.e. hydrophilic poly(oxyethylene) blocks and hydrophobic poly(oxypropylene) blocks, to make up a triblock of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene). The triblock co- polymers absorb water to form gels which exhibit reverse thermal gelation behavior. However, the Pluronic^{R} system is non-biodegradable and the gel properties (water soluble gel) and drug release kinetics (very rapid) from those gels have not proved useful and are in need of substantial improvement. Such a system is described in US Patent No. 6,117,949.

The present invention is based on a mixture of phospholipids of different lengths and concentrations that is a real solution at room temperatures or lower but which forms a semi-solid or gel when warmed to body temperature. When exposed to aqueous environments

the gel gradually erodes, thus releasing compounds/agents trapped therein.

Phospholipid molecules exhibit amphiphilic properties and therefore disperse individually in water when present at low concentrations, yet aggregate to form micelles above a critical concentration. These micelles spontaneously form in aqueous solutions a hydrophobic core and an interfacial polar region. Depending on the lipid concentration, the hydrophobic core of these micelles exhibits a wide range of geometries. The most commonly known geometries include self-closed classical micelles and spherical or oval structures, referred to as liposomes, where one or several phospholipid bilayers entraps part of the solvent in its interior.

Recently, a new kind of discoidal micelles was characterized [Forrest., B ,J. and Reeves., L, W. (1981) *Chemical reviews*, 81:1-13; Sanders., C, R. and Prosser., R , S. (1998) *Structure*, 6:1227-1234]. These discoidal micelles are believed to be aqueous lipid-detergent assemblies in which discrete bilayer fragments are edge stabilized by certain detergents. These bicelles (coined for bilayered micelles) are somewhat of an intermediate between classical mixed micelles and lipid vesicles exhibiting optical transparency and no compartmentalization characteristic of classical micelles, yet maintain key bilayer properties. It may be mentioned that bicelles were originally developed for NMR studies of membrane bound or water soluble molecules and complexes, their importance stemming from their ability to undergo alignment in an external magnetic field. Bicelles usually orient with their director perpendicular to the magnetic field as shown in Figure 2.

Bicelles are most commonly prepared from mixtures of dimyristoyl-phosphatidylcholine (DMPC) with either dihexanoylphosphatidylcholine (DHPC) or the bile salt derivative 3-(cholamidopropyl)-dimethylammonio- 2-hydroxy-1-propane sulfonate (CHAPSO). However, other detergents including glycocholate, glycodeoxycholate and conjugate salts of taurine (sodium taurocholate for example) may be used. Bicelles may contain phosphatidylcholines other than DMPC such as dilaurylphosphatidylcholine (DLPC), 1-palmitoyl-2-oleoyl-glycero-phosphatidylcholine (POPC) and egg yolk phosphatidylcholine (EYPC). These mixtures usually contain a total lipid (phospholipids and detergents) concentration of about 4% to 50% W/V at molar ratios of from about 5:1 to 2:1 between the phospholipids and detergents.

Mixtures of DMPC, or other suitable long chain phosphatidylcholines, with the appropriate detergents, are believed to form disk shaped aggregates, in which the long chain phosphatidylcholine forms an L_α type bilayer section, surrounded by a rim of detergent that isolates the hydrophobic side chains from contact with water. A schematic representation of a bicelle and its components is shown in Figure 3.

The liquid crystal L_α property and the general morphology of the bicelles are maintained over a wide range of lipid to detergent ratios, water content, buffer pH and ionic strength. At given lipid to detergent ratios and total lipid concentration, the mixture can change from liquid to semi-solid gel with temperature increase. Below the melting temperature the mixture is a clear and lucid liquid that can easily be handled with a syringe or pipette. Above the melting temperature the mixture converts to a semi-solid clear gel. This change is related to the liquid crystal-gel (order-disorder) transition of the lipid, for example long chain phosphatidylcholine comprising the bilayer.

For example, in a binary system comprising DMPC and DHPC, a thermo- sensitive phase is functional at DMPC:DHPC ratios of about 3.5:1 to 2:1 and total lipid concentrations ranging from about 3 to 40% w/v.

As may be seen in Figure 4, without DHPC or at very low water content an insoluble, lamellar phase incapable of thermal transition is formed (A). At proper DMPC: DHPC ratios and water content a thermo-sensitive discoidal phase is formed (B). At excess DHPC or water an isotropic discoidal thermo-insensitive phase is formed (C). At greater water contents the disks break down to classical mixed micelles and mono-dispersed phospholipids (not shown).

For a 2.5:1 (lipid:detergent) sample, when the water content exceeds 60% w/v the mixture becomes isotropic and looses its ability to form the semi-solid gel upon heating. As the water content increases the bicelles start to diminish in size until they break down to their phospholipid components.

By adjusting the total lipid concentration and the phosphatidylcholine to detergent ratio, it is possible to predetermine the T.m of the system. It is thus possible to fit the system to a variety of conditions.

It is possible to introduce a large variety of hydrophobic and hydrophilic compounds into the bicellar system. This can be simply achieved by dissolving the desired compound, in dry form, in the liquid phase at temperatures below the T.m or by using a solution containing the desired compounds as the aqueous phase when preparing the bicelles. When heated above the T.m, the entire system, including the introduced compounds turns to a semi-solid gel.

For example, for increasing the stability of the bicellar phase, the anionic lipid 1,2-dimyristoyl-*sn*-3-phosphoglycerol (DMPG) or the cationic lipid 1,2-dimyristoyl-3-trimethylammoniumpropane (DMTAP) can be added. DMPG/DMPC ratios may range up to 50/50 and DMTAP/DMPC ratios from up to 40/60, while the overall concentration of DHPC remains constant. The formation of bicelles containing charged amphiphiles is contingent upon the presence of NaCl, with 50 mM NaCl being sufficient for bicelle formation.

In order to increase the stability of bicelles over a wide pH range, it is possible to replace the diacyl phospholipid groups of DMPC and DHPC with their hydrolysis resistant dialkyl analogs. Mixtures of 1,2-O-ditetradecyl-sn-glycero-phosphocholine (ditetradecyl-phosphatidylcholine) and 1,2-O-dihexyl-*sn*-glycero-phosphocholine (dihexyl-phosphatidylcholine) in water form lyotropic liquid crystalline phases under conditions similar to those previously reported for bicelles consisting of DMPC and DHPC [Ottiger., M. and Bax., A. (1999) *Journal of Biomolecular NMR* 13:187-191].

Another possible mixture in which the carboxyl ester sites have been replaced by the considerably more acid-stable ether derivative is a bicelle system composed of 1,2-O-di-dodecyl-*sn*-glycerophosphocholine and CHAPSO, at molar ratios of around 4.3 to 1.

Thus, the bicellar matrix of the delivery system of the invention may comprise any suitable mixture of lipid-detergent in an aqueous medium, and additionally, such substances that increase the stability of the bicelles, the stable pH range and/or optimize magnetic properties, as the substances described above.

At the proper lipid:detergent ratios (e.g., phosphtidylcholine:detergent ratio) and total lipid concentrations, the melting temperature can be set to body temperature. This enables easy internal or external application of the cooled mixture, which turns to gel on contact with body tissues. As the mixture gels, a localized depot is formed and maintains its position in the tissue (see Examples 2, 4). As the gel is exposed to living tissue, it begins to break down due the higher water content thus liberating the compounds trapped therein.

The rate of gel decomposition can be predetermined in the preparatory stage by adjusting the lipid (phosphatidylcholine):detergent ratio and total lipid concentration. Further control of the rate of release of trapped compounds can be achieved by introducing negatively or positively charged molecules to the bicellar phase. The charged molecules can be either charged phospholipids or any other charged molecule incorporated into the bicelles themselves at desired concentrations in order to electrostatically control the rate of release. Such additives have been discussed above. The adjustment of the rate of release of the active agent and/or of the rate of erosion of the depot is within the skills of the man of the art of pharmacy.

In addition, the rate of decomposition can be determined by the targeted tissue, depending on the moisture and surface area.

Being comprised of phospholipids and naturally occurring or mild detergents, the system is biocompatible and its degradation products non-toxic.

The advantages of the bicellar system of the invention as a delivery system are, including but not limited to:

(1) For application to the skin, the drug delivery system can be applied to tender, sensitive skin by a pour-on or spray application with no need to rub the area to obtain coverage.
(2) For parenteral applications, the delivery system can be introduced by the simple means of a syringe.
(3) Local administration of a pharmaceutical agent to the specific tissue in need of such treatment significantly reduces the required doses in comparison to systemic administration dose:body weight ratio.
(4) Once introduced to the desired target location, the delivery system gels and remains at the location, gradually decomposing and releasing the trapped substances.
(5) The rate of decomposition can be determined by the total lipid concentration, long chain phosphatidylcholine:detergent ratio and targeted tissue.
(6) The system is biocompatible and its degradation products non-toxic products.

Thus, in a preferred embodiment, the delivery system of the invention can be used for the sustained release of parentally administered compounds including, but not restricted to peptides and proteins, anticancer agents, antibacterial substances, anti-infective agents, anti-inflammatory drugs, antiviral agents, anesthetics, antifungal agents, antiparasitic agents, analgesics, smoking crave suppressants, drugs for male and female sexual disturbances, depilatory compounds and DNA and RNA.

Thus, for example, a delivery system containing a carcinostatic or cytostatic drug may be used for local treatment of solid tumors, by injection to the tumor or its close vicinity. Such mode of

administration would both enhance the efficacy of treatment and reduce systemic side effects associated with chemotherapy.

In another embodiment of the invention the system can be used for providing local radiotherapy. This object can be achieved by using a bicellar system in which at least part of the lipid or detergent are radiolabled to an extent sufficient to provide therapeutically effective radioactive radiation. For example, phosphatidylcholine may be labeled by P^{32}.

Alternatively, the provision of local radiotherapy can be obtained by using a bicellar system in accordance with the invention, comprising as an active agent a radiotherapeutically effective amount of radionuclide/s. Standard radionuclides of iodine, iridium, radium, cesium, yttrium or other elements may be used. Preferred radioisotopes are those which have a particle range in tissue which is concordant with the thickness of the layer of tissue to be treated. Information on particle ranges is readily available [Burnazyan & Lebedinskii, Radiation Medicine, in International Series of Monographs in Nuclear Energy, Pergamon Press, 1964]. For example, it is known that about 90% of the energy from a ^{14}C (carbon-14) source will be absorbed in about the first 70 microns of tissue, and similar distances will be found for ^{35}S (sulfur-35) and ^{33}P (phosphorous-33), since their emitted particles are of the same kind as ^{14}C (beta particles) and of similar energies. More energetic beta particles would have a longer range, such as those of ^{32}P (phosphorous-32), which has a maximum range of about one centimeter, and thus can be used to treat thicker tumors, or blood vessels having multi-millimeter thick medial layers.

In another embodiment of the invention the bicellar phase can be used as an antigenicity enhancing (adjuvant) system. Conventional adjuvants are oil-based and include a suspension of minerals (alum, aluminum hydroxide or phosphate) on which the antigen is absorbed, or water-in-oil emulsion in which antigen solution is emulsified in mineral oil (Freund complete adjuvant), to further enhance antigenicity. The bicellar phase can be an alternative adjuvant, based on its gradual erosion properties. The bicellar phase may optionally contain killed bacteria, for example mycobacteria.

In yet another embodiment of the invention, the system is used for depilation. The size and composition of the bicelles enable efficient absorbency through the epidermis. A depilatory compound, for example sodium or calcium thioglycolate, contained in the bicellar matrix, applied to the skin, can thus be efficiently transported through the epidermis to hair follicles.

Still another embodiment is the use of the delivery system of the invention as a transfecting agent. In this embodiment, the bicellar system comprises as active ingredient a transfecting agent. The transfecting agent can be an one oligo- or polynucleotide, a gene or a plasmid or DNA vector comprising the same. When *in vitro* or *in vivo* contacted with cells, they become efficiently transfected with the nucleic acid. Such system is exemplified in Example 5. Of particular interest are transfecting agents comprising a DNA sequence encoding a therapeutic protein or protein products or itself a therapeutic product, antisense DNA sequences and ribozymes. Such transfecting agents are useful in gene therapy, gene repair and inhibition of gene expression, where desired.

The transfection system in accordance with the invention can be used *ex vivo* or *in vivo*. For *ex vivo* use, cells can be obtained from a patient in need of such treatment or from a compatible donor, transfected with the desired transfecting agent at a temperature equal to or above the gelation temperature of the bicellar matrix, and the transfected cell are then introduced into said subject. For *in vivo* use, the transfection system of the invention is parenterally administered to the subject in need.

Disclosed and described, it is to be understood that this invention is not limited to the particular examples, process steps, and materials disclosed herein as such process steps and materials may vary somewhat. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only and not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise.

The following examples are representative of techniques employed by the inventor in carrying out aspects of the present invention. It should

be appreciated that while these techniques are exemplary of preferred embodiments for the practice of the invention, those of skill in the art, in light of the present disclosure, will recognize that numerous modifications can be made without departing from the intended scope of the invention.

Examples

Example 1: Sustained release of toluidine blue

Control Assessment: Two ml of an aqueous solution containing 1 mM toluidine blue and 50 mM NaCl were placed in a dialysis tubing (2000 molecular weight cut off). The ends of the tubing were secured using a rubber band. The tubing was placed into a 250 ml beaker containing 100 ml of a 50 mM NaCl solution. A stir bar was added and the beaker was placed on a stir plate in a 37^{o}C incubator.

Aliquots of 1 ml were periodically removed from the beaker and the absorbance at 634 nm was measured. The samples were returned to the beaker following optical reading. This procedure was repeated several times over a 2 hour time period. The temperature of the solution in the beaker was also determined at each pull.

Test Sample evaluation:

A bicellar phase containing DMPC and DHPC at a 2.7:1 molar ratio and total lipid concentration of 25% (w/v) was prepared by dissolving the appropriate amount of lipids in an aqueous solution containing 25 mM Hepes-K pH 7 and 25 mM NaCl. The melting temperature of this bicellar phase is around 25^{o}C.

400 µl of 5 mM toluidine blue were added to 1.6 ml of liquid bicellar phase and placed in a dialysis tubing (2000 molecular weight cut off).

The tubing was placed in an air tight bag and set into a 37-40^{o}C water bath to allow the sample to gel. The dialysis tubing containing the gelled sample was placed in a 250 ml beaker containing 100 ml of a 50 mM NaCl solution. A stir bar was added and the beaker was placed on a stir plate in a 37^{o}C incubator.

Aliquots of 1 ml were periodically removed from the beaker and the absorbance at 634 nm was measured. The samples were returned to the beaker following optical reading. This procedure was repeated several times over a 4 hour time period. The temperature of the solution in the beaker was also determined at each pull.

Figure 1 depicts the release profile of toluidine blue entrapped in the gelled bicellar phase (circles) versus the simple diffusion of the dye from an aqueous solution (squares).

As is evident from Figure 1, the release rate of the dye from the bicellar system is considerably slower than the release from the aqueous solution.

In the control experiments the concentration of dye in the dialyzing solution increased gradually until approaching complete release after an hour and a half, while exhibiting a release profile governed by simple diffusion.

In the bicellar system, only about half of the entrapped dye was released in a period of 4 hours. Furthermore, the linear phase of the release from the bicellar system suggests that surface erosion, rather than simple diffusion, is responsible for the sustained release of the entrapped compound.

It is expected that a bicellar phase placed in a dialysis bag would form an extended non spherical structure as opposed to the spherical gel depots observed when introducing the system intramuscularly (see following examples). As the geometry influences the rate of surface erosion, it is most likely that the rate of release from a bicellar depot introduced intramuscularly may be much slower than the rate determined in this *in vitro* test and may very well extend to release periods of weeks if not months.

Example 2: The ability of the gelling phase to sustain whole cells *in vitro*

A bicellar phase containing DMPC and DHPC at a 2.5:1 molar ratio and total lipid concentration of 30% (w/v) was prepared using Complete Tissue Culture Medium (CTCM) as solvent. The mixture was sonicated and taken through several freeze-thaw cycles until a homogenous clear sample was obtained. The mixture was kept on ice and in sterile conditions until used.

Splenocytes were harvested from the spleen of a 6 week old C57BL/6 mouse and added into tubes containing cold CTCM (denoted CTCM-A) or CTCM + liquid bicellar phase (denoted CTCM-B), at a concentration of $5x10^6$ cells/ml. The tubes content (with splenocytes) was divided into several wells containing 200µl each ($1x10^6$ cells/well) and maintained at 37° C in a 5% CO_2 incubator. The CTCM-B samples changed consistency from liquid to semi-solid state immediately after entering the 37°C environment. Two, and 24 hours following incubation the CTCM-A and CTCM-B wells were put on ice for liquefaction and samples were taken for light-microscopy observation.

Cell counts conducted for the CTCM-A and CTCM-B samples showed no significant difference. Cells present in the CTCM-B wells survived

re-entry to the incubator and subsequent liquefaction, indicating that changes from liquid to semi-solid have no lytic effect and are reversible.

Example 3: The ability of the gelling phase to remain at its inoculation site *In vivo*

Cold aliquots of 0.150 ml CTCM-B and CTCM-A were injected subcutaneously to C57BL/6 mice. Once under the skin, CTCT-B changed to a semi-solid form that was easily palpable. CTCM-A injected to control mice was rapidly absorbed by the tissue and left no residual signs. Observations by palpation, carried out daily, confirmed the continuous presence of semi-solid CTCM-B at the same location. On the 10^{th} day, the mice were sacrificed and skin tissues surrounding the injected location were analyzed. Inspection of the CTCM-B injected mice revealed a round fibrotic tissue surrounding the area of injection. As formation of fibrotic tissue requires more than 12 hours, this would indicate that the subcutaneously injected CTCM-B did not dissolve immediately, as the CTCM-A samples did, but rather remained at its original location for some time.

Example 4: Toxicity Studies

To test the possible toxicity of the delivery system of the invention, a bicellar system ("test article") comprising DMPC and DHPC, at a molar ratio of 2.7: 1, respectively and 25% (w/v) total lipid was prepared and applied to the three tests described bellow.

Systemic Toxicity Testing:

Test Methodology: Groups of healthy albino male and female mice (17-23gr) were injected intramuscularly (IM) with 0.1 ml of the test article or subcutaneously (SQ) with 0.2 ml of the test article. All mice were

injected at a rate of 0.1 ml/sec. Saline was used as a control and was injected into separate groups of animals in the same manner. The animals were observed for signs of toxicity immediately after injection and at 4, 24, 48 and 72 hours post injection. At that time, half of the mice were euthanized. The remaining mice were observed daily for a total of fourteen days after injection.

Results: The mice evaluated did not exhibit any signs of systemic toxicity and did not experience significant weight loss after injection (where significant weight loss is defined as loss of greater than 2 grams.)

Agar Diffusion Cytotoxicity Test:

Test Methodology: Cell cultures (cell line L929) were grown to a standard monolayer (plate size 60mm), overlaid with 7 ml of agar, and stained with natural red before treatment. The test article was placed on the agar surface via a filter paper disk (Cold, aqueous solution, 0.1 ml). Approximately 10% of the cell agar layer were subjected to this treatment. After 24 hours of incubation (at 37°C and 5% CO_2), the cells were scored microscopically for decolorization and lysis, to determine biological reactivity of the test article compared to positive and negative control samples.

Results: No cytotoxic reaction was observed

Primary Skin Irritation Test:

Test Methodology: Rabbits were shaved (dorsal region) a day before testing. Three healthy, albino, white rabbits weighing not less than 2 kg ((NZw)SPF) were used to evaluate the test article directly with intact skin. The skin of additional 3 rabbits was abraded and the test sample was applied to the abraded sites as well. The test article was

placed on patches (0.5 ml per patch), that were applied to the skin of the rabbit on either side of the spine. Adjacent areas of skin were treated with a control (water). After 24 hours, the patches were removed and the rabbits were scored for erythema and edema at 24, 48, and 72 hours post patch removal. The test scores were subtracted from the control scores to achieve the Primary Irritation Score (PIS). The Primary Irritation Index was determined by averaging the scores for all animals.

Results: Based on the Primary Irritation Index the test article produced a slight irritation response, an acceptable value for medical device biocompatibility assessment.

Biocompatibility; Test Summary:

The following tests: the Systemic Toxicity Test, the Agar Diffusion Cytotoxicity Assay and the Primary Skin Irritation Test, were performed to assess the toxicity of the test article. The results of each of the tests indicate that the test article meets the test requirements.

The tests were conducted at STS Duotek Inc. (7500 West Henrietta Road, Rush, New York, 14543), reviewed and approved.

Procedural References:

(1) 1996 Association for the Advancement of Medical Instrumentation, Volume 4; Biological Evaluation of Medical Devices ANSI/AAMI/ISO 10993-10-1995

(2) Federal Register (CFR) Volume 50, Number 188, p. 39429, "Rules and Regulations"; September 27, 1990.

(3) Sterilization Technical Services, Standard Operating Procedures for "Primary Skin Irritation ISO Method" (SST-2.1)

(4) Association for the Advancement of Medical Instrumentation (AAMI) Standards and Recommended Practices, 1997 Edition, Volume 4: Biological Evaluation of Medical Devices – Part 5: Tests for Cytotoxicity: *In vitro* methods (Identical to ISO 10993-5:1192).

(5) United States Pharmacopeia/ National Formulary, Current Edition.

(6) STS Standard Operating Procedures CYT-1.

(7) "Annual Book of ASTM Standards: Medical Devices," American Society for Testing and Materials, Section 13, Volume 13.01, 1996 and subsequent revisions.

(8) FDA Class III Contact Lenses and Solutions, April 1989 and July 1985, respectively.

(9) Sterilization Technical Services, Standard Operating Procedures for "Systemic Injection Test".

(10) Chan, P.K., O'Hara, G.P. and Hayes, A.W.: "Principles and Methods for Acute and Subchronic Toxicity" Principles and Methods of Toxicology; Raven Press, New York, New York, 1982.

(11) STS Standard Operating Procedures CYT-1.

Example 5: *In vivo* transfection

The following example was carried out with the pcDNA3/Alkaline Phosphatase (AP) marker gene (7.3 kbp). A bicellar system comprising DMPC and DHPC, at a molar ratio of 2.7:1, respectively, and 35% (w/v) total lipid was prepared. Stearylamine (SA) was added to the bicelles in amounts that resulted in a charge ratio of 1:3 or 1:6 between the pDNA and the SA. The pDNA solution was added to cold bicelle solution (10% w/v of DNA:bicelles), and mixed gently while the bicelles were placed on ice.

Six-weeks-old Sprague Dawley male rats were anaesthetized by ether and randomly assigned. The tibial area was shaved, and pDNA (50 μg

pDNA/200 µl saline), pDNA-Bicelles 1:3 (containing 50 µg pDNA), or pDNA-Bicelles 1:6, were longitudinally injected into the muscle using a 1 ml sterile syringe fitted with a 25G 5/8" needle. The control group received either saline or blank bicelles. Three days after pDNA administrations, the tibial muscle was removed, homogenized, and incubated for 15 min. with 1ml DNA lysis buffer.

The lysed supernatant was heated to 65°C for 30 minutes in order to inactivate the endogenous AP (the recombinant AP is stable under these conditions). A specific chemiluminescence assay kit (Tropix, Bedford, MA, USA) was used to determine the recombinant AP levels. Results were normalized to total protein content determined by BCA total protein assay (Pierce, Rockford, IL, USA), and are expressed as light units (LU) per mg protein.

AP expression in rat tibialis muscle 3 days after a single IM injection is shown in Figure 5. Treatment with pDNA-Bicelles 1:3 and 1:6 resulted in significant gene expression compared to the control. A charge ratio of 1:3 resulted in higher AP levels than the 1:6 ratio.

An important finding, stressing the applicability of intramuscular introduction of the described system, is that no adverse tissue reaction was observes at the site of injection. It should be noted that the bicellar depot was present at the injection site 3 days following the injection, indicating that a longer time scale may be necessary for full decomposition.

When comparing the results of the bicellar phase experiment with those of the naked DNA, it appears that the naked DNA has given better AP expression. However, it is to be appreciated that being a sustained release system, the amount of DNA released from the

bicellar phase was significantly smaller than the amount delivered as naked DNA (most of the bicellar material was found intact at the site of injection, when the animals were sacrificed). Considering that the bicellar matrix releases the DNA in a sustained manner, much higher levels of AP expression levels would be obtained after a period time longer than the 3 days of this particular experiment. These higher levels of expression are expected to be higher than the expression measured after 3 days for naked DNA transfection.

Claims:

1. A sustained release delivery system for the delivery of an active agent to a warm-blooded animal comprising:

a. an aqueous bicellar matrix that is liquid at temperatures below ambient temperature and forms a biodegradable gel at body temperature; and

b. an active agent;

and optionally further comprising pharmaceutically acceptable additive, carrier and/or diluent.

2. A sustained release delivery system of claim 1, comprising:

a. an aqueous bicellar matrix that is liquid at temperatures below ambient temperature and forms a biodegradable gel at body temperature;

b. an active agent; and

c. an additive being a pH adjusting agent

and optionally further comprising pharmaceutically acceptable carrier and/or diluent.

3. A sustained release delivery system of claim 1 or claim 2, wherein said matrix comprises a mixture of a lipid, preferably phospholipid, and a detergent in water.

4. A sustained release delivery system of claim 3, wherein said lipid is a long chain phosphatidylcholine.

5. A sustained release delivery system of claim 4, wherein said long chain phosphatidylcholine is selected from the group consisting of dimyristoylphosphatidylcholine (DMPC) and hydrolysis-resistant dialkyl analogs thereof such as 1,2-O-ditetradecyl-sn-glycero-phosphocholine or 1,2-O-di-dodecyl-sn-glycero-phosphocholine.

6. A sustained release delivery system of claim 4, wherein said long chain phosphatidylcholine is dimyristoylphosphatidylcholine (DMPC).

7. A sustained release delivery system of any one of claims 3 to 5, wherein said detergent is selected from the group consisting of dihexanoylphosphatidylcholine (DHPC), and hydrolysis-resistant dialkyl analogs thereof such as 1,2-O-dihexyl-sn-glycero-phosphocholine.

8. A sustained release delivery system of any one of claims 3 to 7, wherein said detergent is dihexanoylphosphatidylcholine (DHPC).

9. A sustained release delivery system of claim 8, wherein the total lipid concentration is in the range of about 3% to 40% w/v, and the molar ratio between DMPC and DHPC is in the range of from 4:1 to 1.5:1.

10. A sustained release delivery system of any one of claims 3 to 6, wherein said detergent is 3-(cholamidopropyl)dimethylammonio- 2-hydroxy-1-propane sulfonate (CHAPSO).

11. A sustained release delivery system of claim 10, wherein the total lipid concentration is in the range of about 5% to 40% w/v, and the molar ratio between DMPC and CHAPSO is in the range of from 5:1 to 2:1.

12. A sustained release delivery system of any one of claims 3 to 6, wherein said detergent is glycocholate.

13. A sustained release delivery system of claim 12, wherein the total lipid concentration is in the range of about 45% to 65% w/v, and the molar ratio between DMPC and glycocholate is in the range of from 2:1 to 8:1.

14. The sustained release delivery system of any one of claims 1 to 13, further comprising positively or negatively charged phospholipids.

15. The sustained release delivery system of claim 14, wherein the concentration of said charged phospholipids is about 0.01%-5% w/v.

16. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is a peptide or protein selected from the group consisting of oxytocin, vasopressin, adrenocorticotropic hormone, epidermal growth factor, platelet-derived growth factor (PDGF), prolactin, luliberin ,luteinizing hormone releasing hormone (LHRH), LHRH agonists, LHRH antagonists, growth hormone (human, porcine, bovine, etc.), growth hormone releasing factor, insulin, somatostatin, glucagon, interleukin-2 (IL-2), interferon-alpha, -beta, or -gamma, gastrin, tetragastrin, pentagastrin, urogastrone, secretin, calcitonin, enkephalins, endorphins, angiotensins, thyrotropin releasing hormone (TRH), tumor necrosis factor (TNF), nerve growth factor (NGF), granulocyte-colony-stimulating factor (G-CSF) granulocyte-macrophage-colony-stimulating factor (GM-CSF), macrophage- colony-stimulating factor (M-CSF), heparinase, bone morphogenic protein (BMP), hANP, glucagon-like peptide (GLP-1), interleukin- 11 (IL-11), renin, bradykinin, bacitracins, polymyxins, colistins, tyrocidine, gramicidins, cyclosporins, enzymes, cytokines, antibodies and monoclonal antibodies and pharmaceutically active synthetic analogues, derivatives, modifications and fragments thereof.

17. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is an anticancer agent selected from the group consisting of mitomycin, bleomycin, BCNU, carboplatin, doxorubicin, daunorubicin, methotrexate, paclitaxel, taxotere, actinomycin D, and camptothecin.

18. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is an antibacterial substance selected from the group consisting of beta-lactam antibiotics, tetracyclines, chloramphenicol, neomycin, gramicidin, bacitracin, sulfonamides, aminoglycoside antibiotics, tobramycin, nitrofurazone, nalidixic acid and analogs, the antimicrobial combination of fludalanine/pentizidone, nitrofurazone, cephalosporins, chlortetracyclin, clindamycin, erythromycins, gentamicin, ofloxacin, penicillins and streptomycin.

19. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is a bacteriostatic agent selected from the group consisting of iodine, chloramines, benzalkonium chloride and phenol.

20. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is an steroidal or non-steroidal anti-inflammatory drug selected from the group consisting of cortisone, hydrocortisone, betamethasone, dexamethasone, fluocortolone, prednisolone, triamcinolone, indomethacin, sulindac and its salts and corresponding sulfide.

21. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is an antiparasitic agent selected from

the group consisting of ivermectin, tetrahydropyrimidine, benzimidazol, quinoline and molevac.

22. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is an antiviral agent selected from the group consisting of acyclovir, interferon, amantadine, famciclovir, valacyclovir, oseltamivir, ganciclovir, idoxuridine, vidarabine, trifluridine.

23. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is an anesthetic selected from the group consisting of benzocaine, lidocaine and dibucaine.

24. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is an antifungal agent selected from the group consisting of tolnaftate, undecylenic acid, salicylic acid, zinc undecylenate and thiabendazole, amphotericin, butenafine, ciclopiroxolamine, clotrimazole, econazole, flucomazol, fungilin, galivert, heralvent, oricant, grape fruit seed extract, hylac forte, ketoconazole, miconazole, mucokehl, mutaflor, paidoflor, naftifine, natamycin, nystatin, omnifloran, pefrakehl, prosymbioflor, symbioflor 1 and 2, taheebo, trenev-trio.

25. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is an analgesic agent selected from the group consisting of methylsalicylate, menthol, camphor, methylnicotinate, triethanolamine salicylate, glycol salicylate and salicylamine, salsalate, phenacetin, p-aminosalicyl acid, diflunisal, salicylate combinations, tramadol, propoxyphene, morphine sulfate, hydromorphone, meperidine, pentazocine and pentazocine combinations, and the combinations: codeine

phosphate/acetaminophen, propoxyphene HCl/aspirin, propoxyphene HCl/acetaminophen, hydrocodone bitartrate/aspirin, hydrocodone bitartrate/ acetaminophen, hydrocodone/ibuprofen, oxycodone/acetaminophen and oxycodone/aspirin.

26. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is a radionuclide selected from the group consisting of iodine, iridium, radium, cesium and yttrium.

27. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is a depilatory agent.

28. The sustained release delivery system of any one of claims 1 to 15, wherein said active agent is a transfecting agent selected from the group consisting of plasmids and/or polynucleotides.

29. A radiotherapeutic composition for providing radioactive therapy to a warm-blooded animal comprising:
a radioactive aqueous bicellar matrix that is liquid at temperatures below ambient temperature and forms a biodegradable gel at body temperature;
and optionally further comprising pharmaceutically acceptable additive, carrier and/or diluent.

30. The composition of claim 29, comprising an additive being a pH adjusting agent, and optionally further comprising pharmaceutically acceptable carrier and/or diluent.

31. The composition of claim 29 or claim 30, wherein said matrix comprises a mixture of a lipid, preferably phospholipid, and a

detergent in water, wherein all or part of any of said lipid or detergent is radioactively labeled.

32. The composition of claim 31, wherein said lipid is a long chain phosphatidylcholine.

33. The composition of claim 32, wherein the long chain phosphatidylcholine is dimyristoylphosphatidylcholine (DMPC).

34. The composition of any one of claims 31 to 33, wherein said detergent is dihexanoylphosphatidylcholine (DHPC).

35. The composition of claim 34, wherein the total lipid concentration is in the range of about 3% to 40% w/v, and the molar ratio between DMPC and DHPC is in the range of from 4:1 to 1.5:1.

36. The composition of any one of claims 31 to 33, wherein said detergent is 3-(cholamidopropyl)dimethylammonio-2-hydroxy-1-propane sulfonate (CHAPSO).

37. The composition of claim 36, wherein the total lipid concentration is in the range of about 5% to 40% w/v, and the molar ratio between DMPC and CHAPSO is in the range of from 5:1 to 2:1.

38. The composition of any one of claims 31 to 33, wherein said detergent is glycocholate.

39. The composition of claim 38, wherein the total lipid concentration is in the range of about 45% to 65% w/v, and the molar ratio between DMPC and glycocholate is in the range of from 2:1 to 8:1.

40. The delivery system of any one of claims 1 to 15, wherein said active agent is a vaccinating agent in admixture with an immunogenic agent that elicits an immune response.

41. The delivery system of claim 40, wherein said immunogenic agent is selected from the group consisting of killed bacteria, preferably killed mycobacteria.

42. A method of treating a medical condition in a patient in need of such treatment, comprising parenterally administering to said patient a pharmaceutical composition comprising a bicellar matrix that is liquid at ambient temperature and forms a biodegradable gel upon administration to said patient, a therapeutically effective amount of a drug and a pH adjusting agent, said composition further optionally comprising a physiologically acceptable carrier and/or diluent.

43. The method of claim 42, wherein said matrix comprises an aqueous mixture of a lipid and a detergent.

44. The method of claim 43, wherein said lipid is a long chain phosphatidylcholine.

45. A method of providing radiotherapy to a patient in need of such treatment, comprising parenterally administering to said patient a radiotherapeutic composition comprising an aqueous mixture of a lipid and a detergent, which mixture is liquid at ambient temperature and forms a biodegradable gel upon administration to said patient, a therapeutically effective amount of radionuclide and a pH adjusting agent, said composition further optionally comprising a physiologically acceptable carrier and/or diluent.

46. The method of claim 45, wherein said matrix comprises an aqueous mixture of a lipid and a detergent.

47. The method of claim 46, wherein said lipid is a long chain phosphatidylcholine.

48. A method of providing radiotherapy to a patient in need of such treatment, comprising parenterally administering to said patient a radiotherapeutic composition comprising an aqueous mixture of a lipid, preferably phospholipid, with a detergent, which mixture is liquid at ambient temperature and forms a biodegradable gel upon administration to said patient, a pH adjusting agent, wherein at least one of said lipid and detergent is radioactively labeled, said composition further optionally comprising a physiologically acceptable carrier and/or diluent.

49. The method of claim 49, wherein said matrix comprises an aqueous mixture of a lipid, preferably phospholipid, and a detergent.

50. The method of claim 49, wherein said lipid is a long chain phosphatidylcholine.

51. A method for providing efficient vaccination to a patient in need thereof, comprising parenterally administering to said patient a pharmaceutical composition comprising a bicellar matrix that is liquid at ambient temperature and forms a biodegradable gel upon administration to said patient, an effective amount of an immunogenic agent that elicits an immune response, the vaccinating agent and a pH adjusting agent, said composition further optionally comprising a physiologically acceptable carrier and/or diluent.

52. The method of claim 51, wherein said matrix comprises an aqueous mixture of a lipid, preferably phospholipid, and a detergent.

53. The method of claim 52, wherein said lipid is a long chain phosphatidylcholine.

54. A method of transfecting cells comprising the steps of:
a. providing cells to be transfected;
b. providing a bicellar matrix that is liquid at ambient temperature and forms a biodegradable gel at a temperature above 15°C, said matrix containing a transfecting agent, a pH adjusting agent and optionally physiologically acceptable carrier and/or diluent;
c. contacting said cells with the bicellar matrix provided in step (b) and maintaining the mixture at a temperature above 15°C.

55. The method of claim 54, wherein said bicellar matrix comprises an aqueous mixture of a lipid, preferably phospholipid, and a detergent.

56. The method of claim 55, wherein said lipid is a long chain phosphatidylcholine.

57. The method of any one of claims 55 and 56, wherein said transfecting agent is any one of an oligonucleotide, a polynucleotide, a gene and any DNA vector containing the same.

58. The method of claim 57, wherein said gene encodes a therapeutic protein or protein product or is itself a therapeutic product.

59. A method of in vivo transfecting cells in a patient in need of such treatment, comprising parenterally administering to said patient a bicellar matrix that is liquid at ambient temperature and forms a biodegradable gel at body temperature, said matrix containing a transfecting agent, a pH adjusting agent and optionally physiologically acceptable carrier and/or diluent.

60. The method of claim 59, wherein said bicellar matrix comprises an aqueous mixture of a lipid, preferably phospholipid, and a detergent.

61. The method of claim 60, wherein said lipid is a long chain phosphatidylcholine.

62. The method of any one of claims 60 and 61, wherein said transfecting agent is any one of an oligonucleotide, a polynucleotide, a gene and any DNA vector containing the same.

63. The method of claim 62, wherein said gene encodes a therapeutic protein or protein product or is itself a therapeutic product.

64. A method of removing hair from the skin of a subject, comprising applying to the skin of said subject a pharmaceutical composition comprising a bicellar matrix that is liquid at ambient temperature and forms a biodegradable gel upon contact with the skin, a depilatory agent and a pH adjusting agent, said composition further optionally comprising a physiologically acceptable carrier and/or diluent.

65. The method of claim 64, wherein said matrix comprises an aqueous mixture of a lipid, preferably phospholipid and a detergent.

66. The method of claim 65, wherein said lipid is a long chain phosphatidylcholine.

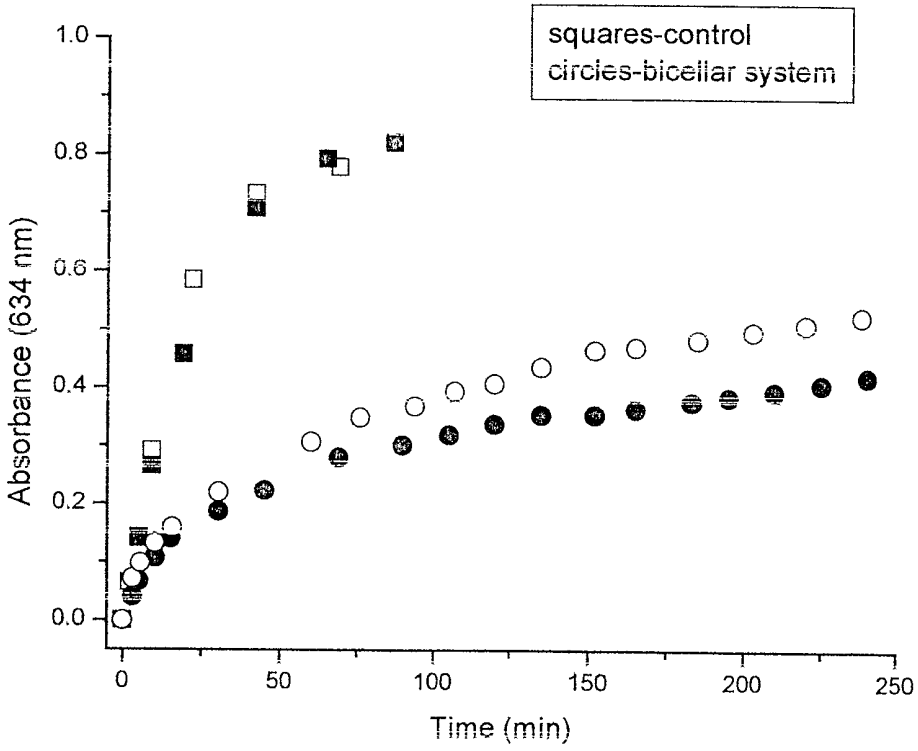
squares-control
circles-bicellar system
Absorbance (634 nm)
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
Time (min)

Figure 1

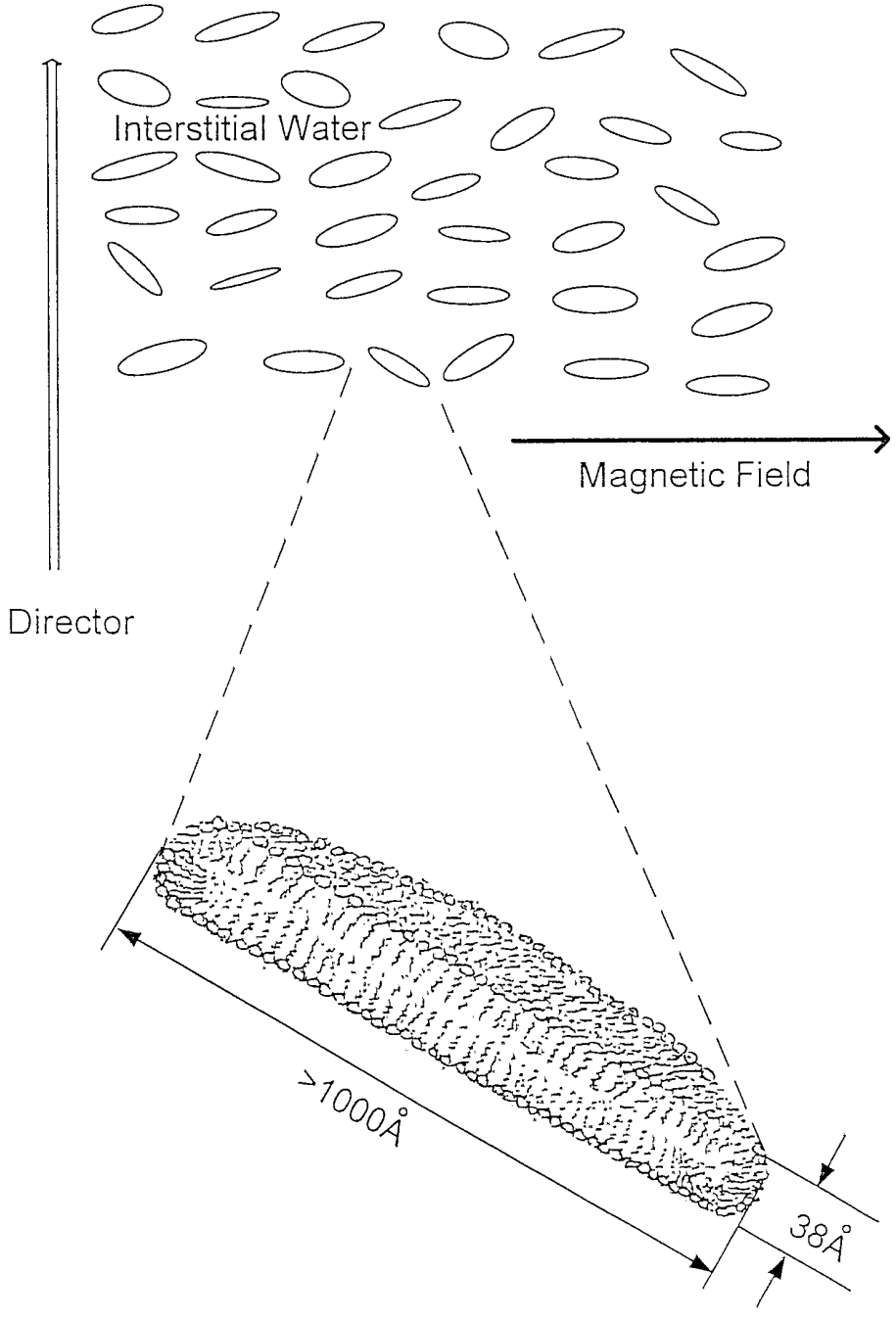

Figure 2

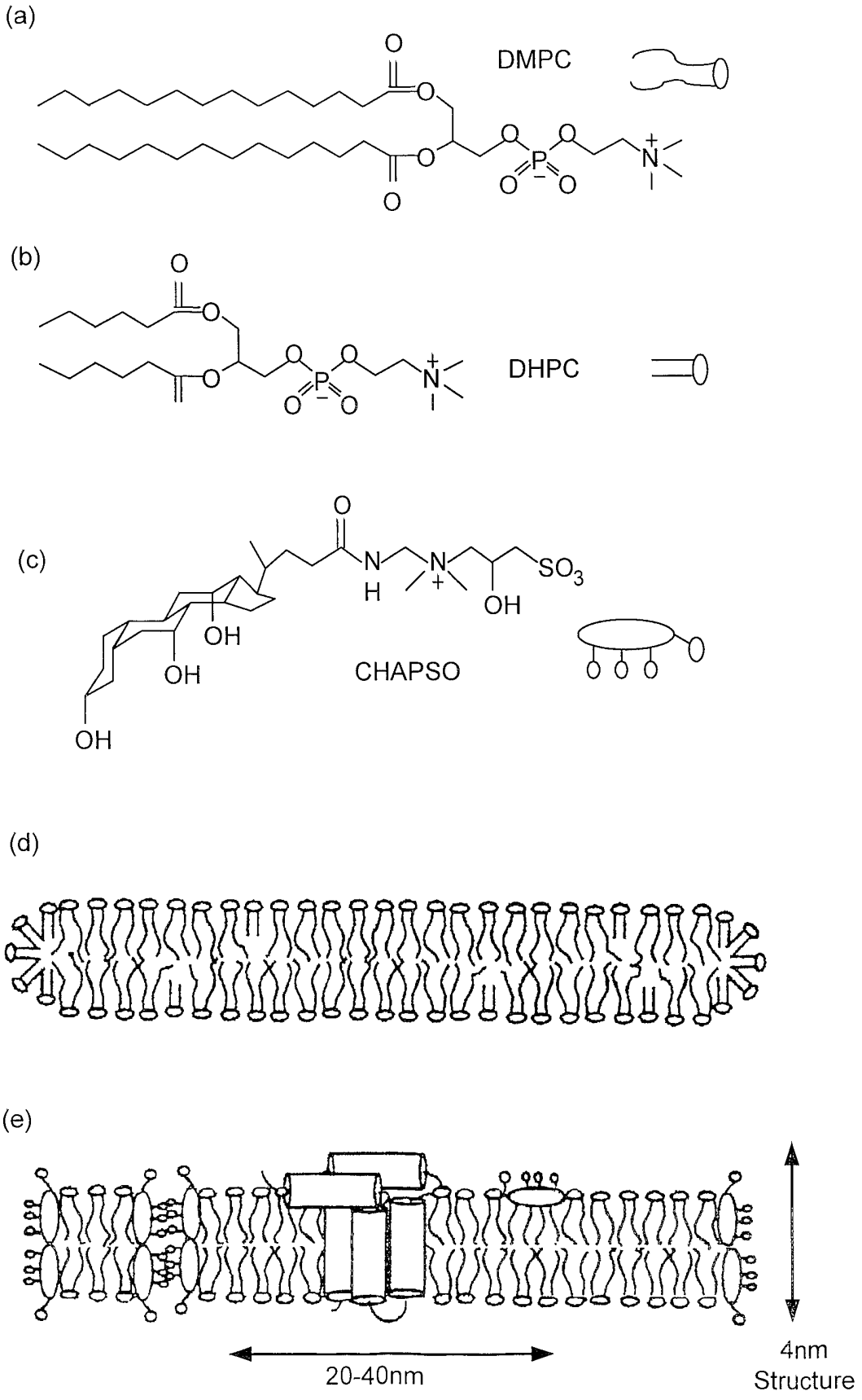

Figure 3

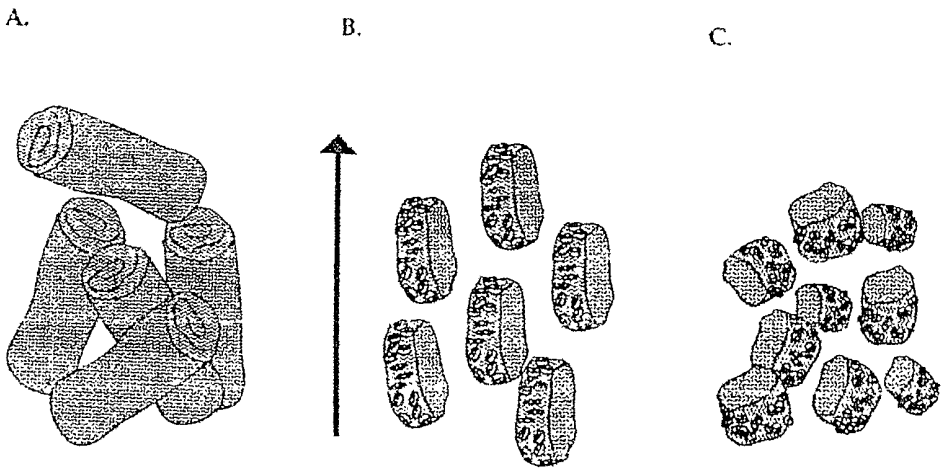

Figure 4

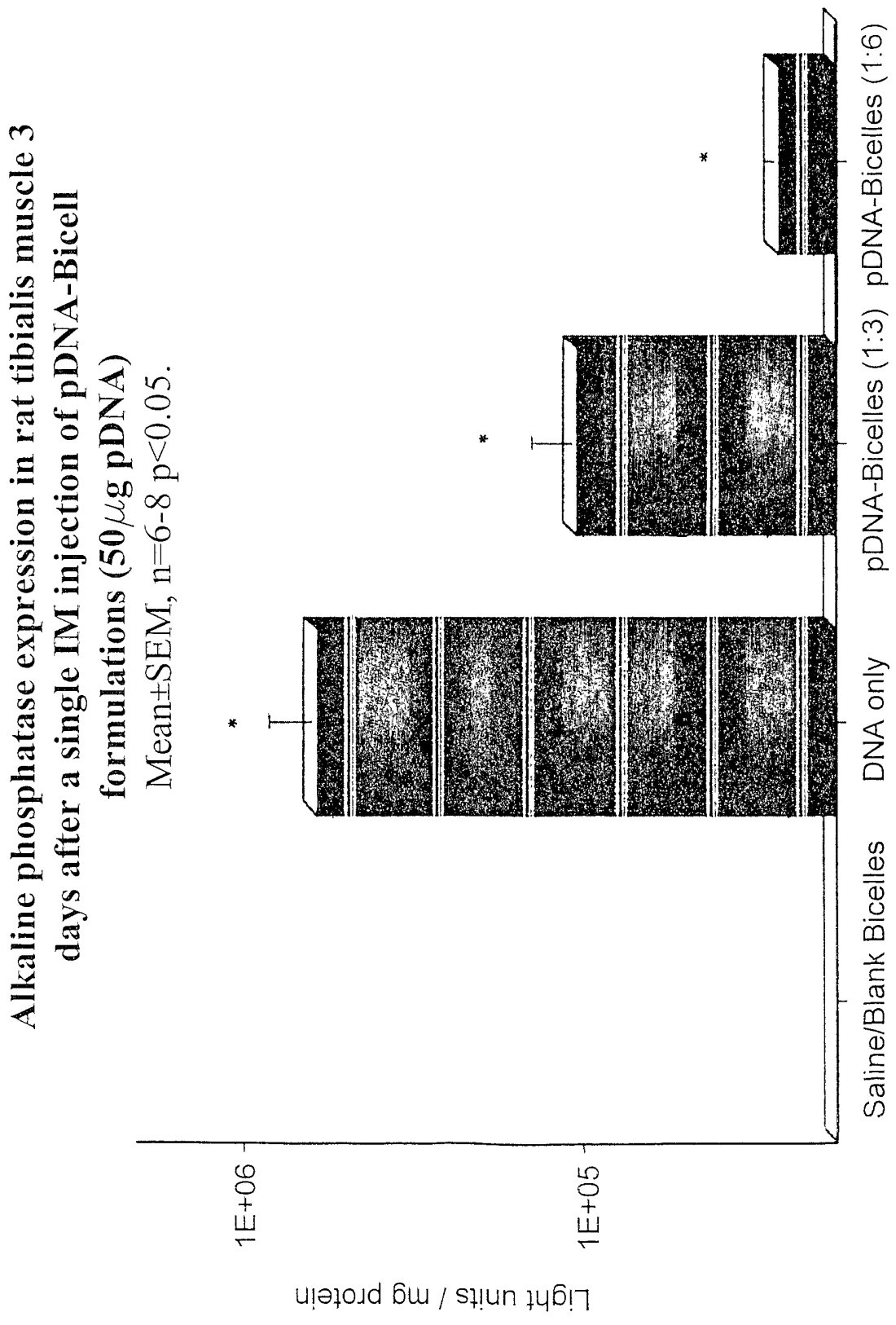
Alkaline phosphatase expression in rat tibialis muscle 3 days after a single IM injection of pDNA-Bicell formulations (50μg pDNA)
Mean±SEM, n=6-8 p<0.05.
Light units / mg protein
1E+06
1E+05
*
*
*
Saline/Blank Bicelles
DNA only
pDNA-Bicelles (1:3)
pDNA-Bicelles (1:6)

Figure 5