HYALURONIC ACID AND HYDROPHOBIC POLY AMINO ACID COPOLYMER

Title:

Abstract:

Provided is a hyaluronic acid-hydrophobic polynmio acid copolymer comprising hyaluronic acid units and hydrophobic polynmio acid units. The hyaluronic acid-hydrophobic polynmio acid copolymer is biodegradable in vivo and can be used for delivery of active ingredients such as organic molecule drugs (including proteins, peptides and nucleotides), food additives and cosmetic ingredients. Particularly when it is used for delivery of drugs, the hyaluronic acid-hydrophobic polynmio acid copolymer is capable of maintaining biological stability of the active ingredient and achieving in vivo sustained release of the active ingredient with providing long-acting effects for one week or more.
HYALURONIC ACID AND HYDROPHOBIC POLY AMINO ACID COPOLYMER

FIELD OF THE INVENTION

The present invention relates to a biodegradable hyaluronic acid-hydrophobic polyamino acid copolymer comprising hyaluronic acid units and hydrophobic polyamino acid units, which can be used for delivery of active ingredients such as organic molecule drugs (including proteins, peptides and nucleotides), food additives and cosmetic ingredients, and is particularly useful for delivery of protein and peptide drugs.

BACKGROUND OF THE INVENTION

Among numerous polymers which are used for delivery of pharmaceutically, sitologically and/or cosmetically active ingredients, the beginning of study on a biodegradable polymer used for \textit{in vivo} delivery of a drug as an active ingredient dates back to an investigation of polylactic acid (PLA) in 1970s.

Since the study of polylactic acid, there are continuing attempts to find polymers which have biodegradable properties thereby result in easy disappearance \textit{in vivo} and which also exhibit non-toxicity and safety of the degraded units upon \textit{in vivo} decomposition of the polymer.
For example, there has been a continuing trend toward the development of various beneficial polymers such as poly(lactic-co-glycolic acid) (PLGA), naturally-occurring polysaccharides, polyamino acids, polyethylene glycol (PEG)-poly(lactic-co-glycolic acid) (PLGA), polyethylene oxide (PEO)-polypropylene oxide (PPO), and the like. Some carrier materials using these polymers are currently commercially available on the market.

Synthetic polymers including PLGA have an advantage of easy in vivo biodegradability due to having ester linkages in molecular structures, but suffer from a potential problem in that the ester linkages discharge hydrogen ions (H\(^+\)) to result in denaturation of protein and peptide drugs, consequently leading to significant deterioration in stability of active ingredients. Therefore, a number of attempts have been made to solve these problems.

In this connection, US Patent No. 6,946,145 discloses a drug delivery method which uses a hydrophilic and lipophilic polyorthoester copolymer containing amine groups in encapsulation or solubilization of sparingly-soluble drugs such as anticancer agents. According to this US patent, the polyorthoester copolymer will spontaneously self-aggregate in an aqueous solution to form micelles, and is employed for sustained release of the drug.

US Patent No. 5,904,936 discloses a drug carrier based on a polyamino acid copolymer comprising at least two types of recurring amino acids and having both hydrophilic and lipophilic properties, wherein the polymer will spontaneously self-aggregate in an aqueous solution to form particles into which target drugs such as proteins are encapsulated and then delivered into the body, followed by gradual release of the drug in the body.
PCT WO 99/018142 discloses a drug delivery system using a thermosensitive polymer which is based on a copolymer of polyethylene glycol (PEG)-polylactic acid (PLA)-glycolic acid (PLGA) blocks that exist as a liquid phase at room temperature but, when the temperature is raised to about body temperature, spontaneously interact to form hydrogels.

US Patent No. 6,800,663 discloses a drug delivery system in which a copolymer of poly(alpha-hydroxy acid-glycidyl methacrylate) and polyethylene glycol is crosslinked to form a hydrogel network, thereby delivering the drug of interest and controlling release of the drug.

Further, Carbohydrate Polymers 69 (2007) p. 597-606 discloses a drug delivery system using nanoparticles of cholesterol-modified 0-carboxymethyl chitosan conjugates in which lipophilic cholesterol of the polymer undergoes spontaneous self-aggregation in an aqueous solution, and a hydrophilic portion of the polymer is negatively charged.

Further, Biomaterials 28 (2007) p. 41324142 discloses in vivo delivery of drugs such as negatively charged nucleotides, via preparation of positively charged micelles in an aqueous solution, using a polyethyleneimine/polycaprolactone copolymer.

As discussed above, even though a variety of polymers have been proposed for in vivo delivery and transport of drugs, these polymers do not sufficiently meet the requirements essential for a drug carrier, i.e. conditions that degradation products of the polymer carrier should be safe to the human body and sustained release of the drug can be appropriately controlled while not inhibiting stability of the drug.

SUMMARY OF THE INVENTION
Therefore, the present invention has been made to solve the above problems and other technical problems that have yet to be resolved.

Specifically, it is an object of the present invention to provide a hyaluronic acid-hydrophobic polyamino acid copolymer, which is capable of achieving effective in vivo delivery of active ingredients and easy in vivo degradability of the copolymer and also exhibits non-toxicity and safety of degradation products, as a polymer intended for delivery of pharmaceutically, histologically and/or cosmetically active ingredients, and which is a novel biocompatible and biodegradable material capable of achieving in vivo sustained release of active ingredients.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

FIG. 1 is a graph showing blood insulin levels in rats following administration of fine particles of Example 3 in accordance with the present invention and an insulin solution of Comparative Example 1.

**DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS**

In accordance with an aspect of the present invention, the above and other objects can be accomplished by the provision of a hyaluronic acid-hydrophobic polyamino acid copolymer comprising hyaluronic acid units and hydrophobic polyamino acid units.
Such a hyaluronic acid-hydrøphobic polyamino acid copolymer forms micelles in an aqueous solution, and physical combining of the hydrophobic polyamino acid and active ingredients in the micelles hinders the access of water (H₂O) in vivo to thereby significantly improve sustained release properties of the active ingredient. Further, the copolymer is readily decomposable in vivo due to having biodegradabilily arising from unique characteristics in molecular structures, and can be usefully employed for in vivo delivery and sustained release of active ingredients such as organic molecule drugs (including proteins, peptides and nucleotides), food additives and cosmetic ingredients, and particularly protein and peptide drugs.

The aforesaid copolymer may form various types of copolymers, for example random copolymers, block copolymers, graft copolymers, and the like. Preferred are block copolymers or graft copolymers. More preferred are graft copolymers.

Hyaluronic acid (HA) which is a constituent component of the copolymer is a linear biopolymer consisting of alternating residues of N-acetyl-D-glucosamine and D-glucuronic acid monosaccharide as repeat units. Hyaluronic acid may be extracted and purified from various and diverse organisms and tissues such as vitreous humor, joint synovial fluid, cockscomb, and the like, by a conventional method known in the art, such as acid solubilization, alkaline solubilization, neutral solubilization and enzymatic solubilization. Hyaluronic acid may be prepared to have various ranges of a molecular weight, depending upon extraction and purification methods, determination methods, etc. For the purpose of the present invention, the molecular weight of hyaluronic acid may be preferably 5x10⁵ Da or higher, more preferably 1x10⁶ Da or higher, and particularly preferably 1x10⁶ Da to 3x10⁶ Da.
The polyamino acid, another constituent component of the copolymer, consists of hydrophobic amino acids. These hydrophobic amino acids increase the hydrophobicity of a formulation of interest, which may consequently enhance sustained release properties of drugs such as physiologically active insulin.

As used herein, the term "hydrophobic amino acid" refers to an amino acid having relatively low-water solubility rather than the amino acid having high-water solubility (hydrophilic amino acids). Examples of the hydrophobic amino acid may include, but are not limited to, leucine, isoleucine, methionine, alanine, phenylalanine, tryptophan, valine, and the like. Particularly preferred are leucine, isoleucine, and phenylalanine. These hydrophobic amino acids may be used alone or in any combination thereof.

The hydrophobic polyamino acid may be synthesized from an N-carboxy anhydride (NCA) of the hydrophobic amino acid, and forms a hydrophobic portion of the hyaluronic acid-hydrophobic polyamino acid copolymer.

In one preferred embodiment of the present invention, the hydrophobic polyamino acid may be polyleucine synthesized from an N-carboxy anhydride (NCA) of leucine, polyisoleucine synthesized from an N-carboxy anhydride (NCA) of isoleucine, or polyphenylalanine synthesized from an N-carboxy anhydride (NCA) of phenylalanine.

A dry weight ratio of the hyaluronic acid unit and the hydrophobic polyamino acid unit in the copolymer is in a range of preferably 50:1 to 1:1, more preferably 30:1 to 2:1, and particularly preferably 10:1 to 3:1.
Synthesis of the copolymer may be preferably carried out in an organic solvent. Here, in order to dissolve hydrophilic hyaluronic acid in the organic solvent, hydrophilic groups may be substituted with an organic salt such as a tetrabutyl ammonium (IBA) salt. Thereafter, an N-carboxy anhydride (NCA) of the amino acid is added to the organic solvent thereby induce polymerization of the reactants. The polymerization is initiated by hydroxy (OH) groups of hyaluronic acid. The polymerization of the N-carboxy anhydride (NCA) of the amino acid is well known in the art, so a detailed description thereof will be omitted herein for brevity (see Kricheldorf, α-Aminoacid-N-carboxyanhydrides and Related Heterocycles, Chap.2, pp.51-157, Springer-Verlag, Paris, 1987).

In accordance with another aspect of the present invention, there is provided a composition comprising a hyaluronic acid-hydrophobic polyamino acid copolymer and one or more active ingredients, and a pharmaceutical, sitological or cosmetic composition comprising the same.

There is no particular limit to kinds of the active ingredients, as long as they are intended for in vivo delivery. Examples of the active ingredients may include proteins, peptides, nucleotides, and organic small compounds having hydrophobic or hydrophilic functional group(s).

In one preferred embodiment of the present invention, the active ingredient may be a therapeutically effective amount of insulin.

As used herein, the term "therapeutically effective amount" means an amount of an active ingredient that is effective to relieve or reduce to some extent one or more of the symptoms of the disease in need of treatment, or to retard initiation of clinical markers or symptoms of a disease in need of prevention, when the compound is administered. Thus, a therapeutically effective amount refers to an amount of the active ingredient which exhibit effects of (i) reversing the rate of progress
of a disease; (ϋ) inhibiting to some extent further progress of the disease; and/or, (iii) relieving (or, preferably, eliminating) to some extent one or more symptoms associated with the disease. The therapeutically effective amount may be empirically determined by experimenting with the compounds concerned in known in vivo and in vitro model systems for a disease in need of treatment.

Insulin is a polypeptide hormone having a very short half-life of about 30 min, and therefore exhibits very high absorption and disappearance rates in vivo. For this reason, diabetic patients have suffered from discomfort and inconvenience associated with frequent self-injection of insulin more than three times a day, such that the blood insulin concentration is maintained at a constant level.

On the other hand, the hyaluronic acid-hydrophobic polyamino acid copolymer in accordance with the present invention undergoes spontaneous formation of fine particles between the hydrophobic portions in an aqueous solution, due to impartment of hydrophobicity to the hydrophilic hyaluronic acid by the polyamino acid. In this connection, the hydrophobic polyamino acid and insulin are combined by physical bonds other than by chemical bonds, such structural characteristics inhibit the access of water (H₂O) in vivo to thereby significantly improve the stability and sustained release properties of the insulin drug. Accordingly, it is advantageously possible to minimize discomfort and inconvenience of patients which may occur due to administration of insulin.

The fine particles may be of a spherical, non-spherical or irregular shape, and may preferably have an injectable size of 1 to 500 µm.
In order to improve sustained release properties of the active ingredient, it is possible to
obtain further improved synergistic sustained release effects when the hyaluronic acid-polyamino
acid copolymer is contained in an amount that is capable of inducing sustained release and
absorption of the active ingredient.

As used herein, the phrase "amount that is capable of inducing sustained release and
absorption of the active ingredient" means a sufficient amount that is capable of inhibiting release
and uptake rates of the active ingredient into the blood. Therefore, the hyaluronic acid/hydrophobic
amino acid copolymer may be contained in an amount of 50 to 99.9% by weight, preferably 70 to
99% by weight, and more preferably 90 to 95% by weight, based on the total dry weight of the
composition,

In one preferred embodiment of the present invention, the pharmaceutical composition
may be a particulate formulation comprising i) 90 to 95% by weight of the hyaluronic acid-
polyleucine copolymer consisting of hyaluronic acid having a molecular weight of $10^6$ Da and a
leucine N-carboxy anhydride (NCA) in a dry weight ratio of 10:1 to 3:1, and ii) a therapeutically
effective amount of insulin as an active ingredient, üi) wherein the insulin is physically combined
with the hyaluronic acid-polyleucine copolymer in an aqueous solution.

When the composition in accordance with the present invention is used as a sitological or
cosmetic composition, a sitologically acceptable carrier or cosmetically acceptable carrier may be
further added to the composition.

For example, the composition containing the sitologically acceptable carrier may be used
as a health food or otherwise may be added thereto. As used herein, the term "health food" refers to

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a food in which the composition of the present invention is added to a general food to thereby improve functions thereof. For this purpose, the composition of the present invention may be added to general foods or may be prepared in the form of capsules, powders, suspensions and the like. Intake of such a health food containing the composition of the present invention provides advantages in that there are no adverse side effects which may occur upon long-term administration of drugs because a food is used as the raw material, unlike conventional drugs.

If it is desired to use the composition of the present invention as a food additive, the composition can be added alone, or otherwise may be used in conjunction with other foods or food ingredients, or may be used appropriately according to any conventional method. A mixed amount of the active ingredient may be suitably determined depending upon the desired uses and applications (prophylactic, health or therapeutic treatment).

There is no particular limit to kinds of the above-mentioned foods. As examples of foods to which the composition of the present invention can be added, mention may be made of meats, sausages, breads, chocolates, candies, snacks, confectionaries, pizzas, Ramen, other noodles, gum, dairy products including ice creams, various soups, beverages, teas, drinks, alcoholic beverages and multi-vitamin preparations.

The composition of the present invention may be administered via various routes.

As used herein, the term "administration" means an introduction of a certain material into a patient via any suitable method. Administration of the composition of the present invention may be carried out via any conventional administration route, as long as the drug can be delivered to a target tissue. Without being limited thereto, the composition of the present invention may be
formulated into an injectable, transdermal or oral preparation, such that it can be administered via various routes such as intraperitoneal, intravenous, intramuscular, subcutaneous, intradermal, oral, transnasal, intraocular, topical, intranasal, intrapulmonary, and intrarectal administrations. However, due to digestion of insulin upon oral administration, the oral composition has a disadvantage in that there is a need for protective coating of the active drug or a formulation of the active drug to be protected from decomposition thereof in the stomach. Therefore, the active drug may be preferably administered in the form of an injectable formulation. More preferred is subcutaneous administration. Further, the composition of the present invention may be administered via any means through which the active ingredient can migrate toward target cells.

Meanwhile, the composition of the present invention may optionally comprise a pharmaceutically acceptable carrier depending upon administration routes, and may also comprise a stabilizing agent in order to increase stability of the active ingredient. Particularly, when the active ingredient is insulin which is labile, that is, is readily susceptible to aggregation or decomposition, as is well known, the composition may comprise the stabilizing agent capable of increasing the stability of insulin. Such a stabilizing agent is a material which can covalently or non-covalently link to the active ingredient. Examples of the stabilizing agent that can be used in the present invention may include, but are not limited to, sugars such as sucrose, lactose and glucose, polyols such as mannitol and glycerol, surfactants such as polysorbate, preservatives such as cresol, phosphates, inorganic salts, and the like. These stabilizing agents may be used alone or in any combination thereof. Kinds and amounts of the stabilizing agent suitable for insulin may be appropriately selected by those skilled in the art.
In accordance with a further aspect of the present invention, there is provided a sustained-release injectable formulation where the composition of the present invention is dispersed in a solution for injection.

As the solution for injection, aqueous solutions for injection, such as distilled water for injection and buffer for injection, may be preferably used. Where appropriate, the solution for injection may further comprise additional components such as a buffer, a pH adjusting agent, an isotonic agent, a dispersant, an antiseptic, an analgesic, a preservative, and the like.

A suitable dose of the pharmaceutical composition in accordance with the present invention may vary depending upon various factors such as symptoms of the disease in need of treatment, administration routes, age, sex and weight of patients, and severity of disease, in conjunction with kinds of drugs as an active ingredient.

In accordance with yet another aspect of the present invention, there is provided a method for enhancing the in vivo sustainability of a therapeutically active ingredient, through the inclusion of the aforesaid composition (see FIG. 1).

For example, an injectable formulation comprising fine particles of a hyaluronic acid-polyleucine copolymer/insulin conjugate (see Example 4) exhibits significantly excellent sustained-release properties, as compared to an injectable preparation containing insulin alone (see Comparative Example 1).

EXAMPLES
Now, the present invention will be described in more detail with reference to the following Examples. These examples are provided only for illustrating the present invention and should not be construed as limiting the scope and spirit of the present invention.

Preparation Example 1: Preparation of tetrabutylammonium salt of hyaluronic acid

3 g of hyaluronic acid having a molecular weight of $10^6$ Da was dissolved in 300 mL of an aqueous 5% tetrabutyl ammonium hydroxide solution. The resulting solution was dialyzed against a dialysis membrane (molecular weight cut off (MWCO) 12,000) in excessive purified water for 18 hours, and the residual tetrabutyl ammonium hydroxide not replaced was removed. The dialyzed solution was freeze-dried for 3 days.

Example 1: Preparation of hyaluronic acid-polyleucine copolymer

1 g of a tetrabutyl ammonium salt of hyaluronic acid was stirred and dissolved in 200 mL of dimethylsulfoxide at 60°C. 0.2 g of a leucine N-carboxy anhydride was dissolved in 20 mL of a toluene, and added to a hyaluronic acid/tetrabutyl ammonium solution, followed by reaction at 60°C for 18 hours. 100 mL of a 4 M sodium chloride solution and 300 mL of ethanol were added to thereby precipitate the reactants. The reactants were washed with 1 L of ethanol, and centrifuged at 3000 rpm for 10 min to recover a reaction product. Then, vacuum drying was carried out for 2 hours to remove ethanol. The thus-dried reaction product was dissolved in 100 mL of purified water and the resulting solution was dialyzed against a dialysis membrane (MWCO 12,000) in excessive purified water for 18 hours to remove sodium chloride. The dialyzed hyaluronic acid-polyleucine copolymer solution was freeze-dried for 3 days to give a hyaluronic acid-polyleucine copolymer. A dry weight of the product was 1 g (yield: 83%).
Example 2: Preparation of hyaluronic acid-polyphenylalanine copolymer

A hyaluronic acid-polyphenylalanine copolymer was prepared in the same manner as in Example 1, except that 0.1 g of a phenylalanine N-carboxy anhydride was used instead of 0.2 g of a leucine N-carboxy anhydride. A dry weight of the product was 1 g (yield: 91%).

Example 3: Preparation of fine particles of insulin/hyaluronic acid-polyleucine copolymer

20 mg of insulin was dissolved in 12 mL of a 0.01 M hydrochloric acid solution. An acidity of the insulin solution was adjusted to a pH of 7.4 using a 1N sodium hydroxide solution. 200 mg of a hyaluronic acid-polyleucine copolymer was dissolved in the insulin solution, and fine particles were prepared which consist of physical combination of insulin with the hyaluronic acid-polyleucine copolymer.

Comparative Example 1: Preparation of insulin solution

20 mg of insulin was dissolved in 12 mL of a 0.01 M hydrochloric acid solution. An acidity of the insulin solution was adjusted to a pH of 7.4 using a 1N sodium hydroxide solution.

Experimental Example 1: Size measurement of insulin/hyaluronic acid-polyleucine copolymer fine particles

A size of fine particles of the insulin/hyaluronic acid-polyleucine copolymer prepared in Example 3 was measured by a light scattering method (Mastersizer 2000, Malvern Instruments). The results thus obtained are given in Table 1 below.

[Table 1]
Experimental Example 2: IH-NMR analysis of hyaluronic acid-polyleucine copolymer

In order to confirm a leucine peak and a grafting degree of polyleucine for the hyaluronic acid-polyleucine copolymer prepared in Example 1, IH-NMR spectroscopy was carried out. The results thus obtained are given in Tables 2 and 3 below.

<table>
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<tr>
<th>Leucine peak</th>
<th>Chemical shift</th>
<th>Integration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃</td>
<td>0.91</td>
<td>6</td>
</tr>
<tr>
<td>CH₂CH₂</td>
<td>1.62</td>
<td>3.38</td>
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</table>

[Table 3]

<table>
<thead>
<tr>
<th>HA</th>
<th>Chemical shift</th>
<th>Integration</th>
<th>Graft ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unreacted N-CH₃</td>
<td>2.20</td>
<td>8</td>
<td>66</td>
</tr>
<tr>
<td>Reacted N-CH₃</td>
<td>2.56</td>
<td>4.04</td>
<td>34</td>
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</table>

As shown in Tables 2 and 3, it can be seen that polyleucine is bound in a ratio of about 30% for total units of hyaluronic acid.

Experimental Example 3: Measurement of degree of association between hyaluronic acid-polyleucine copolymer and interferon alpha
The association degree of interferon alpha with respect to a varying weight ratio of the hyaluronic acid-polyleucine copolymer prepared in Example 1 was measured using size exclusion HPLC (SEC-HPLC). The results thus obtained are given in Table 4 below.

[Table 4]

<table>
<thead>
<tr>
<th>Weight ratio of hyaluronic acid-polyleucine:interferon alpha</th>
<th>Association degree (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:1</td>
<td>96.3</td>
</tr>
</tbody>
</table>

Referring to Table 4, it can be confirmed that a high association degree of 96.3% is obtained when the weight ratio of the hyaluronic acid-polyleucine copolymer and the active ingredient is 100:1.

Experimental Example 4: Animal experiment for fine particles prepared from insulin and hyaluronic acid-polyleucine copolymer

This experiment is intended to confirm sustained release of insulin following administration of rats with fine particles prepared from insulin and the hyaluronic acid-polyleucine copolymer. Aqueous solutions having an insulin concentration of 1.67 mg/mL were prepared in Example 3 and Comparative Example 1, respectively. The insulin solution of Comparative Example 1 was used as a control group. 0.5 mL of the insulin solution and 0.5 mL of an aqueous fine particle solution formed of insulin and the hyaluronic acid-polyleucine copolymer were each subcutaneously administered to male Sprague-Dawley rats (7 to 8 weeks old), and blood was collected from animals at a time points of 1, 2, 4, 8, and 24 hours. Sera were separated and then blood insulin levels (µU/mL) were measured by ELISA. The results thus obtained are given in FIG. 1.
As shown in FIG. 1, it can be seen that a maximum insulin concentration in blood is lowered in conjunction with a significantly increased half life of insulin in the animal with administration of the particulate formulation of Example 3, as compared to the insulin solution of Comparative Example 1. Therefore, it can be confirmed that the particulate formulation of the present invention formed by combination of the hyaluronic acid-hydrophobic polyamino acid copolymer and insulin exhibits sustained release effects on insulin.

**INDUSTRIAL APPLICABILITY**

As apparent from the above description, a hyaluronic acid-hydrophobic polyamino acid copolymer in accordance with the present invention undergoes spontaneous formation of fine particles in an aqueous solution, which consequently provides *in vivo* sustained release of an active ingredient via physical combination of the active ingredient with the copolymer, and biological stability of the active ingredient *in vivo*.

Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.
WHAT IS CLAIMED IS:

1. A hyaluronic acid-hydrophobic polyamino acid copolymer comprising hyaluronic acid units and hydrophobic polyamino acid units.

2. The copolymer according to claim 1, wherein the molecular weight of hyaluronic acid is \(1 \times 10^6\) Da or higher.

3. The copolymer according to claim 1, wherein the polyamino acid is synthesized from an N-carboxy anhydride (NCA) of a hydrophobic amino acid, and forms a hydrophobic portion of the hyaluronic acid-hydrophobic polyamino acid copolymer.

4. The copolymer according to claim 3, wherein the hydrophobic amino acid is at least one selected from the group consisting of leucine, isoleucine, methionine, alanine, phenylalanine, tryptophan, valine, and any combination thereof.

5. The copolymer according to claim 3, wherein the polyamino acid is polyleucine synthesized from an N-carboxy anhydride (NCA) of leucine, polyisoleucine synthesized from an N-carboxy anhydride (NCA) of isoleucine, or polyphenylalanine synthesized from an N-carboxy anhydride (NCA) of phenylalanine.

6. The copolymer according to claim 1, wherein a weight ratio of the hyaluronic acid and the hydrophobic polyamino acid in the copolymer is in the range of 50:1 to 1:1.
7. The copolymer according to claim 1, wherein the copolymer is prepared by polymerization of organic salt-substituted hyaluronic acid with an N-carboxy anhydride (NCA) of a hydrophobic amino acid in an organic solvent.

8. A composition comprising the hyaluronic acid-hydrophobic polyamino acid copolymer of any one of claims 1 to 7, and one or more active ingredients.

9. The composition according to claim 8, further comprising a pharmaceutically, sitologically or cosmetically acceptable carrier.

10. The composition according to claim 8, wherein the active ingredient is selected from the group consisting of a protein, a peptide, a nucleotide, and an organic small compound having hydrophobic or hydrophilic functional group(s).

11. The composition according to claim 10, wherein the active ingredient is a therapeutically effective amount of insulin.

12. The composition according to claim 8, wherein the hyaluronic acid-hydrophobic polyamino acid copolymer is contained in an amount that is capable of inducing sustained release and absorption of the active ingredient.

13. The composition according to claim 8, wherein a content of the copolymer is in the range of 50 to 99.9% by weight, based on the total dry weight of the composition.

14. The composition according to claim 8, wherein the composition is in the form of fine particles.
15. The composition according to claim 14, wherein the fine particles have an average particle size of 1 to 500 (μM, and provide sustained release of the active ingredient in a solvent.

16. The composition according to claim 8, wherein the composition is formulated into an injectable, transdermal or oral preparation, such that it can be administered via an oral, transnasal, intraocular, subcutaneous, intravenous, intramuscular or intraperitoneal route.

17. A sustained-release injectable formulation comprising the composition of claim 8 dispersed in a solution for injection.

18. A method for enhancing the in vivo sustainability of a therapeutically active ingredient, using the composition of claim 8.
INTERNATIONAL SEARCH REPORT

International application No
PCT/KR2007/005157

A. CLASSIFICATION OF SUBJECT MATTER

A61K 9/22(2006.01)i, A61K 9/32(2006.01)i, A61K 9/52(2006.01)i, A61K 47/30(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation classification (classification system followed by classification symbols)

IPC 8 A61K 9/22, A61K 9/32, A61K 47/00

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)

EKIPASS(KIPO Internal), CAS(ON LINE), PUBMED

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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<tbody>
<tr>
<td>A</td>
<td>EP 0913149 A1 (SSP CO . LTD) 06 May 1999 See abstract and claims 1-10</td>
<td>1-17</td>
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<td>A</td>
<td>KR 102006093300 A1 (LG LIFE SCIENCES LTD ) 24 August 2006 See abstract and claims 1-22</td>
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<td>A</td>
<td>T Ito et al , &quot;Hyaluronic acid and its derivatives as a multi-functional gene expression enhancer Protection from non-specific interactions, adhesion to targeted cells, and transcriptional activation&quot; J Controlled Release, Vol 112, pp 382-388, March 2006 See the whole document</td>
<td>1-17</td>
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</table>

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

*: Special categories of cited documents
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
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  "P" document published prior to the international filing date but later than the priority date claimed

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

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

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

'&' document member of the same patent family

Date of the actual completion of the international search
08 JANUARY 2008 (08 01 2008)

Date of mailing of the international search report
08 JANUARY 2008 (08.01.2008)

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea
Facsimile No 82-42-472-7140

Authorized officer
HAN, Jung Hee
Telephone No 82-42-481-5604

Form PCT/ISA/210 (second sheet) (April 2007)
INTERNATIONAL SEARCH REPORT

International application No
PCT/KR2007/005157

Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

1  [7]  Claims Nos 18 because they relate to subject matter not required to be searched by this Authority, namely

Claim 18 pertains to the method for treatment of the human or animal body by therapy, as well as diagnostic methods, and thus relates to a subject matter which this International Searching Authority is not required to search under Article 17(2)(a)(i) of the PCT and Rule 39 l(iv) of the Regulations under the PCT

2  [ ]  Claims Nos because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

3  [ ]  Claims Nos because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6-4(a)

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1  [ ]  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

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

3  [ ]  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos

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

Remark on Protest  [ ]  The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee

[ ]  The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation

[ ]  No protest accompanied the payment of additional search fees

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