

【DESCRIPTION】**【Invention Title】**

MULTILAMELLA NANOLIPOSOME WHICH CONTAINS SKIN LIPID
COMPONENTS, AND PREPARATION METHOD THEREFOR

5 **【Technical Field】**

[1] The present invention relates to a multilamella
nanoliposome which is biocompatible, has less irritation to
skin, and includes efficiently stabilized efficacious
materials such as a peptide derived from a living body,
10 etc., to be applicable to cosmetics for skin moisturizing
effect, skin aging prevention, etc., and a preparation
method therefor.

【Background Art】

[2] Skin that provides the first barrier to an external
15 environment due to first contact with the outside of a
human body has an aesthetically important effect in
addition to a simple defensive function. The skin consists
of epidermis and dermis, and the most evident changes are
observed in the epidermis depending on skin aging, moisture
20 content, etc., and thus, the epidermis has been a major
object of research for skin aesthetics.

[3] The major component of the skin is lipid, and various
skin components including the lipid constitute a solid skin
barrier for maintaining a skin shape, defending the skin
25 from a foreign environment, etc. Cholesterol, fatty acid,

and ceramide are typical examples of lipids that constitute the skin barrier, and it is known that it is very difficult for a water-soluble component to penetrate into the skin due to the lipid components of the skin barrier and the skin barrier containing the lipid components.

[4] Among various methods for penetrating the water-soluble component into the skin, a method using liposome has been widely used for skin aging prevention, skin moisturizing, wrinkle improvement, etc. The main reason for using the liposome is that the water-soluble component is able to be delivered stably without changing active components included in the liposome, and is able to be delivered into the skin through the liposome, regardless of whether a property of the active component is fat-soluble or water-soluble.

[5] The liposome may be classified into multilamella liposome or unilamella liposome, and the unilamella liposome may be further divided into a large-membrane liposome or a small-membrane liposome depending on a size.

In general, the multilamella liposome may have a size of 400 to 3,500 nm, and may include a component corresponding to about 5 to 15% of the liposome. The large-membrane liposome in the unilamella liposome may have a size of 100 to 1,000 nm and may contain components in about 30 to 65% of the liposome, and the small-membrane liposome

may have a size of 20 to 50 nm and may include components in about 0.5 to 1.0% of the liposome.

[6] The large-membrane liposome may include the largest amount of components, but is unstable, and the small-
5 membrane liposome is stable but has a very small amount of components that are able to be included. On the other hand, the multilamella liposome has a sufficient amount of components that may be included in the liposome and has good affinity to the skin forming a multilayer structure.
10 However, the multilamella liposome has problems in that it is difficult to maintain stability and a penetration property into the skin is not excellent since it has an excessively large size.

[7] A method for penetrating a water-soluble component
15 into the skin through niosome in addition to liposome is also used. The niosome has a double layer structure which has a size of 10 to 200 nm, includes nonionic surfactant and cholesterol as main components, is closed by high-pressure emulsification, or the like. As compared to
20 liposomes that transport components by lipid particles such as phospholipids, the niosome is relatively stable, is easier to be prepared, and has a lower cost, but has disadvantages in that it is easily aggregated or fused and is not biocompatible.

25 [8] Among lipid components used in preparation of the

liposome, non-hydrogenated lecithin is known to be effective in reducing the size of the liposome and enhancing skin absorbency, but has chemical instability resulted from an unsaturated group due to a property of the liposome present in a water dispersion state, and is accompanied by change in color and fragrance due to acidification, and long term stability is deteriorated. On the other hand, hydrogenated lecithin is advantageous in that since the unsaturated group is removed, chemical stability is excellent, and even though it is present in a water dispersion state, acidification, change in fragrance, or the like, does not occur, and thus, long term stability is excellent. Meanwhile, the hydrogenated lecithin has problems in that due to a property that a saturated hydrocarbon group is rigid, it is difficult to prepare a nano-level liposome having a large radius of curvature, and rather the liposome is transferred to a lamellar phase.

[9] To solve these problems or disadvantages, it is necessary to develop a multilamella liposome that is capable of being stable, having a small size, stably collecting various materials, and having excellent biocompatibility and long-term stability.

[10]

[11] [Related Art Document] Korean Patent Laid-Open Publication No. KR 2004-0078500

[12]

【Disclosure】

【Technical Problem】

[13] An object of the present invention is to provide a
5 multilamella nanoliposome that includes skin lipid
components to be capable of being biocompatible, having
excellent skin permeability, and stably collecting various
efficacious materials, and a preparation method therefor,
thereby overcoming problems of a technology using
10 multilamella liposome and completing the present invention.

[14] The present invention may be used as a cosmetic
composition for skin improvement such as skin moisturizing
enhancement, skin aging prevention, etc., and may be used
even in a pharmaceutical industry and in a food industry in
15 addition to the field of cosmetics.

【Technical Solution】

[15] The present inventors recognized the problems of
existing known liposomes, and invented a multilamella
nanoliposome capable of having chemical stability and long-
20 term stability by using a hydrogenated lecithin, capable of
being stably formed into a nanosize by introducing a
polyglyceryl-based surfactant substituted with an acyl
group, and a fatty acid together thereinto, capable of
being biocompatible, and capable of stably collecting an
25 efficacious material.

[16] In one general aspect, a multilamella nanoliposome includes: a polyglyceryl-based surfactant substituted with C₁₆ to C₂₂ acyl group, a hydrogenated lecithin, and a fatty acid, wherein an efficacious material is stably collected.

5 **[17]** In another general aspect, a preparation method for multilamella nanoliposome includes: a) mixing an oil phase component including a polyglyceryl-based surfactant substituted with C₁₆ to C₂₂ acyl group, a hydrogenated lecithin, and a fatty acid; b) performing emulsification by
10 adding the oil phase component to a water phase component; c) performing high-pressure emulsification to form a nanosized liposome; and d) dispersing a thickener, followed by cooling, thereby stabilizing the liposome.

【Advantageous Effects】

15 **[18]** The multilamella nanoliposome of the present invention is biocompatible and has less irritation to skin, is safe, and includes very stably collected efficacious materials, and further, has excellent moisturizing power and skin permeability, thereby being used variously for
20 skin improvement such as skin moisturizing effect, skin aging prevention, skin wrinkle improvement, etc.

【Description of Drawings】

[19] In FIG. 1, (a) to (d) show a structure and a size of each multilamella nanoliposome according to the present
25 invention.

【Best Mode】

[20] Hereinafter, the present invention will be described in detail. Description of known functions and components that may obscure the gist of the present invention will be omitted from the following description and the accompanying drawings. Terms that are not specifically defined in the entire specification may be construed in a conventional sense to be used by those skilled in the art to which the present invention belongs.

[21] The present invention relates to a multilamella nanoliposome including a polyglyceryl-based surfactant substituted with C₁₆ to C₂₂ acyl group, a hydrogenated lecithin, and a fatty acid, and an efficacious material that is stably collected, and a preparation method therefor.

The present invention provides a multilamella nanoliposome which is biocompatible, has excellent skin permeability and skin absorbency, thereby being effective for skin improvement, and a preparation method therefor.

[22] The polyglyceryl-based surfactant substituted with C₁₆ to C₂₂ acyl group included in the multilamella nanoliposome of the present invention has a content of 1 to 15 wt%, preferably 3 to 12 wt%, and more preferably 5 to 10 wt% based on a multilamella nanoliposome dispersion liquid. According to an exemplary embodiment of the present invention, when the content is less than 3 wt%, the

multilamella nanoliposome may not be firmly prepared, and thus, stability over time may be deteriorated. When the content is more than 12 wt%, emulsifying power by the surfactant may be increased, and thus, the multilamella nanoliposome may not be prepared due to formation of fine emulsion.

[23] The number of glycerin added in the polyglyceryl-based surfactant is 6 to 20, preferably 8 to 12, but is not limited thereto, and within the above range, it may be effective to reduce the size of the multilamella nanoliposome and further improve stability. Specifically, the polyglyceryl-based surfactant substituted with C₁₆ to C₂₂ acyl group may include at least any one of polyglyceryl-based stearate, polyglyceryl-based palmitate, and polyglyceryl-based behenate.

[24] In the present invention, lecithin, which is a main component of the phospholipid, may be included as a secondary emulsifier to improve stability and biocompatibility of the multilamella nanoliposome, and to reduce the total amount of the surfactant. The lecithin may be classified into lecithin having an unsaturated group and hydrogenated lecithin in which an unsaturated group is removed by hydrogenating the unsaturated group. In the present invention, it is preferable to use hydrogenated lecithin as the lecithin.

[25] The lecithin is prepared by extraction from soybean or egg, followed by purification, and is a mixture including phospholipids having a fatty acid chain having 12 to 24 carbon atoms, such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, phosphatidic acid, etc. A mixture including phosphatidylcholine, as an unsaturated lecithin in which the fatty acid chain constituting a hydrophobic group of the extracted lecithin has about 0 to about 3 double bonds on average, at a content of 70 to 95% according to the degree of purification may be used. The double bonds of the fatty acid chain present in lecithin are easily oxidized by water or active oxygen while the hydrogenated lecithin is hydrogenated to reduce the number of unsaturated groups, thereby having high chemical stability.

[26] The hydrogenated lecithin has a content of 0.05 to 10 wt%, 0.5 to 6 wt%, preferably 1 to 5 wt%, and more preferably 4 to 5 wt% based on the multilamella nanoliposome dispersion liquid. A preferred content of the hydrogenated lecithin may be important in order to maintain stability, particularly, long-term stability in the multilamella nanoliposome. In addition, at the time of using the hydrogenated lecithin satisfying a specific content range, emulsification may be maintained, and low

temperature stability, high temperature stability, prevention of discoloration, and high satisfaction when used on the skin may be exhibited. According to an exemplary embodiment of the present invention, when the content of the hydrogenated lecithin is less than 1%, the stability of the multilamella nanoliposome may be very low, and when the content of the hydrogenated lecithin is more than 5%, discoloration may very easily occur.

[27] According to the present invention, it is possible to further stabilize the multilayer structure of the multilamella nanoliposome by further including a fatty acid. The fatty acid may be fatty acids having 14 to 24 carbon atoms, preferably 16 to 22 carbon atoms in the carbon chain, but is not limited thereto. For example, the fatty acid may include at least any one of lauric acid, myristic acid, palmitic acid, stearic acid, isostearic acid, oleic acid, linoleic acid, arachidonic acid and behenic acid. The content of the fatty acid is 0.1 to 5 wt%, 1 to 5 wt%, 1 to 2 wt%, and 1.5 to 2.5 wt% based on a multilamella nanoliposome dispersion liquid, but is not limited thereto.

[28] The efficacious material that is collected and stabilized in the present invention is not limited as long as it is capable of being stably collected in the multilamella nanoliposome of the present invention, and is a material exhibiting water-soluble or fat-soluble

properties. Preferably, at least any one of a peptide derived from a living body, ceramide, tocopherol, and hyaluronic acid is collected and stabilized as the efficacious material.

5 **[29]** In the present invention, the peptide derived from a living body refers to a protein existing in vivo found in human blood, saliva, etc., and includes at least one of copper peptide, argireline, palmitoyl oligopeptide, and palmitoyl tetrapeptide that are water-soluble. A content
10 of the peptide derived from a living body is 0.001 to 1 wt%, 0.1 to 0.5 wt%, preferably 0.01 to 0.1 wt%, based on the multilamella nanoliposome dispersion liquid, but is not limited thereto.

[30] In the present invention, as the hyaluronic acid, a
15 high molecular weight (HMW) hyaluronic acid may be used for continuously supplying moisture to the skin, and a low molecular weight (LMW) hyaluronic acid may be used for absorbing moisture into the skin. The high molecular weight hyaluronic acid preferably has a molecular weight of
20 30,000 to 100,000 Daltons, and the low molecular weight hyaluronic acid preferably has a molecular weight of 10,000 Daltons or less. Contents of the high molecular weight hyaluronic acid and the low molecular weight hyaluronic acid are preferably different from each other. The high
25 molecular weight hyaluronic acid preferably has a content

of 0.001 to 0.1 wt% based on the multilamella nanoliposome dispersion liquid, and the low molecular weight hyaluronic acid preferably has a content of 0.05 to 1 wt% based on the multilamella nanoliposome dispersion liquid, but each content thereof is not limited thereto.

[31] In the present invention, tocopherol may further improve skin absorption and provide an antioxidant effect, thereby further improving the skin improvement effect. A content of the tocopherol is 0.001 to 0.01 wt%, 0.01 to 5 wt%, preferably 0.1 to 3 wt% based on the multilamella nanoliposome dispersion liquid, but is not limited thereto.

[32] In the present invention, ceramide is one of skin lipid components, and may improve skin affinity and may further improve stability of multilamella liposome by a combination with the fatty acid. The ceramide may be included as phytosphingosine having a precursor form, and may also include both ceramide and phytosphingosine. A content of the ceramide is 0.1 to 10 wt%, preferably 1 to 5 wt%, based on the multilamella nanoliposome dispersion liquid, but is not limited thereto.

[33] The multilamella nanoliposome of the present invention has a size of 50 to 500 nm, which is smaller as compared to conventional multilamella liposomes, has excellent stability, and includes excellent contents of components that may be included. The multilamella

nanoliposome according to an exemplary embodiment of the present invention may have a multilayer structure including a lamellar structure that includes 5 to 7 layers and has an interlayer spacing of 20 to 50 nm.

5 **[34]** The multilamella nanoliposome of the present invention has a smaller size than those of general multilamella liposomes to thereby have high skin absorbency and permeability, maintain a stable multilayer structure, thereby being excellent in skin affinity. Further,
10 according to an exemplary embodiment of the present invention, an amount of the efficacious material that may be included in the multilamella nanoliposome is about 5 to 15%, and the efficacious materials such as a sufficient physiologically active component, etc., may be collected
15 and delivered.

[35] By using the polyglyceryl-based surfactant substituted with C₁₆ to C₂₂ acyl group, the hydrogenated lecithin, and the fatty acid together in the multilamella nanoliposome of the present invention, biocompatibility and
20 long-term stability may be improved, the size of the multilamella nanoliposome may be prepared to 50 to 500 nm, and the multilamella nanoliposome may be stabilized while sufficiently including the content of the efficacious material that is able to be collected. Further, the
25 content of the polyglyceryl-based surfactant may be

controlled by controlling the content of the hydrogenated lecithin, and thus, it is possible to prepare a liposome which is more biocompatible, has a good feeling in use, and is excellent in long-term stability and prevention of discoloration.

[36] The preparation method for the multilamella nanoliposome of the present invention may include: a) mixing an oil phase component including a polyglyceryl-based surfactant substituted with C₁₆ to C₂₂ acyl group, a hydrogenated lecithin, and a fatty acid; b) performing emulsification by adding the oil phase component to a water phase component; c) performing high-pressure emulsification to form a nanosized liposome; and d) dispersing a thickener, followed by cooling, thereby stabilizing the liposome.

[37] The surfactant substituted with C₁₆ to C₂₂ acyl group in step a) of the preparation method for the multilamella nanoliposome has a content of 1 to 15 wt%, preferably 3 to 12 wt%, and more preferably 5 to 10 wt% based on the multilamella nanoliposome dispersion liquid. According to an exemplary embodiment of the present invention, when the content is less than 3 wt%, the multilamella nanoliposome may not be firmly prepared and thus, stability over time may be deteriorated. When the content is more than 12 wt%, emulsifying power by the surfactant may be increased, and thus, the multilamella nanoliposome may not be prepared due

to formation of fine emulsion.

[38] The content of hydrogenated lecithin in step a) of the preparation method for the multilamella nanoliposome is 0.05 to 10 wt%, 0.5 to 6 wt%, preferably 1 to 5 wt%, more preferably 4 to 5 wt% based on the multilamella nanoliposome dispersion liquid. A preferred content of the hydrogenated lecithin may be important in order to maintain stability, particularly, long-term stability in the multilamella nanoliposome. In addition, at the time of using the hydrogenated lecithin satisfying a specific content range, emulsification may be maintained, and low temperature stability, high temperature stability, prevention of discoloration, and high satisfaction when used on the skin may be exhibited. According to an exemplary embodiment of the present invention, when the content of the hydrogenated lecithin is less than 1%, the stability of the multilamella nanoliposome may be very low, and when the content of the hydrogenated lecithin is more than 5%, discoloration may very easily occur.

[39] The efficacious material such as ceramide, tocopherol, or the like, may be added to the oil-phase component in step a) of the preparation method for the multilamella nanoliposome, and may be mixed at 60 to 80°C and dissolved while mixing. The emulsification in step b) may be performed by a general emulsification method, and a water-

soluble peptide, an efficacious material such as hyaluronic acid, a preservative component may be added to the water phase component, and may also be mixed and dissolved in water at 60 to 80°C.

5 **[40]** The high-pressure emulsification in step c) is a process for forming a nano-emulsion state, wherein a multilamella nanoliposome form having a nanosize that is stable at a micro size is formed, and the high-pressure emulsification is performed at a pressure of 500 to 1500
10 bar at 50 to 60°C using a high-pressure emulsifier. The thickener in step d) may include at least one of guar gum, gellan gum, cellulose, agar, pectin, carbomer, poloxamer, xanthan gum, carrageenan gum, locust bean gum, a plant-based polymer, a microbial polymer, an animal-based polymer,
15 a starch-based polymer, an arginic acid-based polymer, a vinyl-based polymer, a polyoxyethylene-based polymer, a polyoxyethylene polyoxypropylene copolymerization system polymer, an acrylic polymer, and an inorganic water-soluble polymer, and a cooling temperature is preferably from 25°C
20 up to 35°C for the stabilization of the multilamella nanoliposome.

[41] In the preparation method, when the thickener is added before the high-pressure emulsification in step c) is performed, an efficiency of the high-pressure
25 emulsification may be lowered, and thus, a stable

multilamella nanoliposome desired in the present invention may not be prepared. Further, in order to satisfy the desired stability in the present invention, it is preferable that each step of the preparation method is performed by a continuous process.

[42] The multilamella nanoliposome of the present invention may further include a vegetable oil, a plant sterol or a skin softening agent as the oil phase component. The multilamella nanoliposome of the present invention may further include an alcohol, a skin conditioner, a dispersing agent, an emulsifier, a wetting agent, a moisturizing agent or a whitening agent as the water phase component. Further, the multilamella nanoliposome of the present invention may further include a preservative component.

[43] Hereinafter, the present invention will be specifically described with reference to the following Examples for practicing the present invention. However, the present invention should not be limited to or restricted by the following Examples.

[44] [Examples 1 to 8] Preparation of multilamella nanoliposome

[45] Component contents of Examples 1 to 8 and Comparative Examples 1 to 6 are shown in Tables 1 and 2 below.

[46] [Table 1]

		Example 1	Example 2	Example 3	Example 4	Comparative Example 1	Comparative Example 2	Comparative Example 3
Oil Phase Component	Limnanthes Alba Seed Oil	5	5	5	5	5	5	5
	Cetyl Octanoate	5	5	5	5	5	5	5
	Palmitic acid	2	2	2	2	2	2	2
	PEG-5 Rapeseed Sterol	2	2	2	2	2	2	2
	Polyglyceryl-10 Stearate	8	8	8	8	8	8	8
	Hydrogenated Lecithin	5	1	3	4	0.1	7	9
	Ceramide 3B	5	5	5	5	5	5	5
	Phytosphingosine	1	1	1	1	1	1	1
	Tocopherol	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Water Phase Component 2	Propanediol	9	9	9	9	9	9	9
	Potassium Cetyl Phosphate	0.3	0.3	0.3	0.3	0.3	0.3	0.3
	Acetyl Glucosamine	7	7	7	7	7	7	7
	Sodium Hyaluronate	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	LMW Sodium Hyaluronate	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Peptide	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Niacinamide	5	5	5	5	5	5	5
Thickener	Polyacrylate-13*Polyisobutene*Poly sorbate 20	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Preservative Component	Phenoxyethanol	0.4	0.4	0.4	0.4	0.4	0.4	0.4
	Ethylhexylglycerin	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Water Phase Component 1	D.I Water	to	to	to	to	to	to	to
		100	100	100	100	100	100	100
	Total	100	100	100	100	100	100	100

[48] [Table 2]

		Example 5	Example 6	Example 7	Example 8	Comparative Example 4	Comparative Example 5	Comparative Example 6
Oil Phase component	Limnantes Alba Seed Oil	5	5	5	5	5	5	5
	Cetyl Octanoate	5	5	5	5	5	5	5
	Palmitic Acid	2	2	2	2	2	2	2
	PEG-5 Rapeseed Sterol	2	2	2	2	2	2	2
	Polyglyceryl-10 Stearate	3	6	9	12	2	13	16
	Hydrogenated Lecithin	4	4	4	4	4	4	4
	Ceramide 3B	5	5	5	5	5	5	5
	Phytosphingosine	1	1	1	1	1	1	1
	Tocopherol	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Water Phase component 2	Propanediol	9	9	9	9	9	9	9
	Potassium Acetyl Phosphate	0.3	0.3	0.3	0.3	0.3	0.3	0.3
	Acetyl Glucosamine	7	7	7	7	7	7	7
	Sodium Hyaluronate	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	LMW Sodium Hyaluronate	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Peptide	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Niacinamide	5	5	5	5	5	5	5
Thickener	Polyacrylate-13*Polyisobutene*Polysorbate 20	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Preservative Component	Phenoxyethanol	0.4	0.4	0.4	0.4	0.4	0.4	0.4
	Ethylhexylglycerin	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Water Phase component 1	D.I Water	to 100	to 100	to 100	to 100	to 100	to 100	to 100
	Total	100	100	100	100	100	100	100

[50] The multilamella nanoliposomes of Examples 1 to 8 and Comparative Examples 1 to 6 were prepared by the following methods. The oil phase components were uniformly mixed and dissolved at 70°C. The water phase component 2 and the preservative component were added to the water phase component 1, and mixed and dissolved at 70°C. After mixing and dissolving, the oil phase components were slowly added and homomixed with the water phase components to perform emulsification. Then, the resultant mixture was subjected to high-pressure emulsification in a high pressure emulsifier at 55°C and 1000 bar to prepare a nanoemulsion. After the high-pressure emulsification, a thickener was added to the prepared nanoemulsion, homomixed, completely dispersed, and cooled to 30°C, thereby obtaining each stabilized multilamella nanoliposome.

[51]

[52] Comparison of stability and particle size according to the time point at which thickener is added

[53] The thickener was required to be added after the high-pressure emulsification. When the thickener was added before the high-pressure emulsion, the multilamella nanoliposome having desired stability and size could not be obtained. The stability test and the average size of the multilamella nanoliposomes prepared in the same manner as in Examples 1 to 8 were measured except that the thickener

was added before the high-pressure emulsification. Experimental results of the size, and experimental results of the stability performed at 45°C for 14 days of the respective multilamella nanoliposomes obtained by varying the time point at which the thickener was added were shown in Table 3. It was confirmed that the respective multilamella nanoliposomes were uniformly formed and stably maintained.

[54]

10 **[55]** [Table 3]

Stability at 45°C and particle size according to the time point at which thickener is added

	Addition before high-pressure emulsification	Addition after high-pressure emulsification
Stability at 45°C for 14 days	Upper layer part separated.	Good
Average particle size	10 μm	100 nm

[56]

[57] Comparison of stability for each surfactant

15 combination

[58] Examples 1 to 4 had the contents of the hydrogenated lecithin as 1, 3, 4 and 5 wt%, respectively, and Comparative Examples 1 to 3 had the contents of the

hydrogenated lecithin as 0.01, 7 and 9 wt%, respectively. Examples 5 to 8 had the contents of the polyglyceryl-based surfactant as 3, 6, 9 and 12 wt%, respectively, and Comparative Examples 4 to 6 had the contents of polyglyceryl-10 stearate which was the polyglyceryl-based surfactant as 2, 13, and 16 wt%, respectively. For comparison of low-temperature stability of Examples 1 to 8 and Comparative Examples 1 to 6, the experiment was performed by storage at -17°C for 1 day and thawing for 1 day, and for high-temperature stability, the experiment was performed by checking at 45°C and 60°C every day. The refrigeration stability of Comparative Example 1 was excellent for 1 week, but in view of high-temperature stability, in particular, when stored at 60°C for 2 days or more, emulsification was broken and stability was decreased. Further, in Comparative Examples 2 and 3, discoloration occurred at a high temperature, and the feeling in use was not good due to an excessive content of lecithin. As results of the stability evaluations of Examples 1 to 4 and Comparative Examples 1 to 3, the stability of the multilamella nanoliposomes including lecithin having a content of 1 to 5 wt% was excellent, but the stability was not good when the content of the lecithin was out of the range of 1 to 5 wt%. In addition, in Table 4, Examples 1 and 4 among the Examples had very good evaluation results

of stability and discoloration, and when the lecithin content was 4 to 5%, the stability and discoloration of the multilamella nanoliposomes were particularly excellent. In Comparative Example 4, the refrigeration stability was excellent for 1 week, but when stored at both 45°C and 60°C for 2 days or more, emulsification was broken and stability was decreased. In Comparative Examples 5 and 6, the multilamella nanoliposomes were not normally prepared. As results of the stability evaluations of Examples 5 to 8 and Comparative Examples 4 to 6, it was confirmed that when the content of the polyglyceryl-based surfactant was out of the range of 3 to 12 wt%, the stability was not good or the multilamella nanoliposomes were not normally prepared. The experimental results of the stability and discoloration were as shown in Table 4 below.

[59] [Table 4]

	Stability	Discoloration
Example 1	5	5
Example 2	4	5
Example 3	4	5
Example 4	5	5
Example 5	5	5
Example 6	5	5
Example 7	4	5

Example 8	5	5
Comparative Example 1	1	3
Comparative Example 2	2	1
Comparative Example 3	2	1
Comparative Example 4	1	4
Comparative Example 5	Not prepared	4
Comparative Example 6	Not prepared	4
1: Bad, 2: Slightly bad, 3: Moderate, 4: Slightly good, 5: very good		

[60]

[61] Confirmation of formation of multilamella liposome

[62] The multilamella nanoliposomes were prepared by using the components according to Examples 1 to 8, and microstructures of the liposomes were confirmed. About 5 to 7 layers of lamellar structure was formed in a multilayer form, and had a length of about 200 nm, wherein the layers were relatively and regularly formed with an interlayer spacing of 20 to 50 nm, and the liposomes had a size of 50 to 500 nm (FIG. 1). When the multilamella nanoliposomes were prepared by using the components according to Comparative Examples 5 and 6, a number of fine emulsions of 20 to 40 nm were prepared, and thus, nanoliposomes having a multilayer form were not prepared.

15 **[63]**

[64] Stability test

[65] The multilamella nanoliposomes prepared by the preparation method of Examples 1 to 8 were used to evaluate the stability on 10 subjects through *in vitro* patch test, and results were shown as in Table 5.

5 **[66]**

[67] [Table 5]

Test Material	Degree of Stimulation	Mean Stimulation Score
(-)control	(-)	0.00
0.3% SDS	++	6.55
Examples 1 to 4	(-)	0.00
(-) : No response / Stimulation score 0 : Faint and mild erythema / Stimulation score 0.5± : Weak erythema with clear border / Stimulation score 1+ : Clear erythema, papule and phlyctenule / Stimulation score 2++ : Severe erythema, bulla / Stimulation score 6+++ : Scab / Stimulation score 12		

[68]

[69] A moisturizing power test was performed using a probe for measuring moisturizing of ARAMO-TS (Aram Huvis Co., Ltd., Korea) instrument, and transepidermal water loss (TEWL) was measured on forearms of 10 subjects using an AquaFlux. (Biox, UK) instrument, thereby measuring average moisturizing power. The results were as shown in Table 6 and Table 7.

15 **[70]**

[71] [Table 6]

Test Material	Before Treatment	Day 1	Day 5	Day 15	Day 20	Day 30
Examples 1 to 4	33.40	+8.60	+5.90	+3.63	+5.73	+6.47

[72] It was confirmed that the moisturizing power was increased by 8 or higher in just one day after the liposome was used, and even on Day 30 after the liposome was continuously used, the moisturizing power was improved by 6.47 as compared to that before treatment.

[73]

[74] [Table 7]

Test material	Before treatment	Day 1	Day 5	Day 15	Day 20	Day 30
Examples 1 to 4	12.64	-7.13	-2.48	0.14	-1.8	-0.48

[75] It could be confirmed that the average moisture loss value, i.e., transepidermal water loss (TEWL) value through the skin was decreased by 7 or more in just one day as compared to that before treatment.

[76]

【CLAIMS】**【Claim 1】**

A multilamella nanoliposome comprising:

a polyglyceryl-based surfactant substituted with C₁₆

5 to C₂₂ acyl group,

a hydrogenated lecithin, and

a fatty acid,

wherein an efficacious material is stably collected.

10 **【Claim 2】**

The multilamella nanoliposome of claim 1, wherein the efficacious material is at least any one of a peptide derived from a living body, hyaluronic acid, ceramide, and tocopherol.

15

【Claim 3】

The multilamella nanoliposome of claim 1, wherein the polyglyceryl-based surfactant substituted with C₁₆ to C₂₂ acyl group includes at least one of polyglyceryl stearate, polyglyceryl palmitate, and polyglyceryl behenate.

20

【Claim 4】

The multilamella nanoliposome of claim 1, wherein the hydrogenated lecithin has a content of 1 to 5 wt% based on a multilamella nanoliposome dispersion liquid.

25

【Claim 5】

The multilamella nanoliposome of claim 1, wherein the polyglyceryl-based surfactant substituted with C₁₆ to C₂₂ acyl group has a content of 3 to 12 wt% based on a multilamella nanoliposome dispersion liquid.

【Claim 6】

The multilamella nanoliposome of claim 2, wherein the peptide derived from a living body includes at least one of copper peptide, argireline, palmitoyl oligopeptide, and palmitoyl tetrapeptide.

【Claim 7】

The multilamella nanoliposome of claim 1, wherein the multilamella nanoliposome has a size of 50 to 500 nm.

【Claim 8】

The multilamella nanoliposome of claim 1, wherein a lamellar structure of the multilamella nanoliposome is a multilayer structure including 5 to 7 layers and having an interlayer spacing of 20 to 50 nm.

【Claim 9】

A cosmetic composition for skin moisturizing

comprising:

the multilamella nanoliposome of any one of claims 1 to 8.

5 **【Claim 10】**

A preparation method for multilamella nanoliposome comprising:

a) mixing an oil phase component including a polyglyceryl-based surfactant substituted with C₁₆ to C₂₂ acyl group, a hydrogenated lecithin, and a fatty acid;

b) performing emulsification by adding the oil phase component to a water phase component;

c) performing high-pressure emulsification to form a nanosized liposome; and

15 d) dispersing a thickener, followed by cooling, thereby stabilizing the liposome.

【Claim 11】

The preparation method of claim 10, wherein the hydrogenated lecithin in step a) has a content of 1 to 5 wt% based on a multilamella nanoliposome dispersion liquid.

【Claim 12】

The preparation method of claim 10, wherein the polyglyceryl-based surfactant substituted with C₁₆ to C₂₂

acyl group in step a) has a content of 3 to 12 wt% based on a multilamella nanoliposome dispersion liquid.

【Claim 13】

5 The preparation method of claim 10, wherein the high-pressure emulsification is performed at 50 to 60°C and 500 to 1500 bar.