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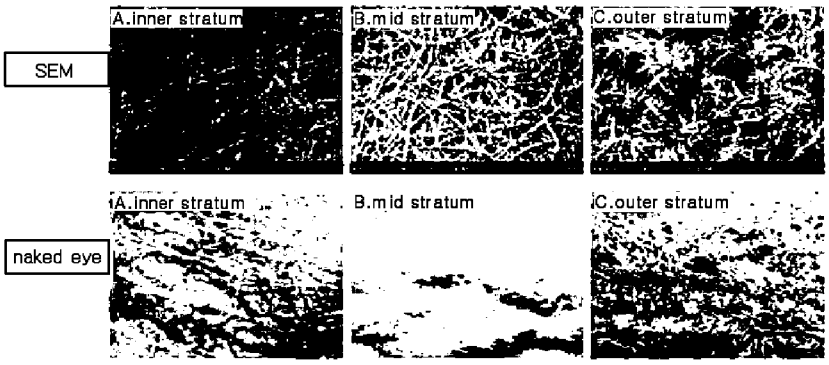
(54) Title: ARTIFICIAL BIOMEMBRANE USING COCOON AND METHOD FOR MANUFACTURING SAME



(57) Abstract: Disclosed herein are a cocoon-based artificial biomembrane and a method for manufacturing the same. A cocoon the shell of which has a first thickness is divided into two or more fragments in a predetermined form. The cocoon fragments may be used as artificial biomembranes. They can be relatively simply manufactured in a more cost efficient manner than conventional artificial biomembranes and have excellent cell growth potential. Also contemplated are a cocoon-based artificial biomembrane having excellent tensile strength and elongation, and a method for manufacturing the same.

Description

Title of Invention: ARTIFICIAL BIOMEMBRANE USING COCOON AND METHOD FOR MANUFACTURING SAME

Technical Field

[1] The present invention relates to an artificial biomembrane using cocoon, and a manufacturing method thereof. More particularly, the present invention relates to a cocoon-based, artificial biomembrane that is biocompatible and has excellent cell growth potential, and a method for manufacturing the same.

[2] Also, the present invention relates to a biomembrane that has excellent tensile strength and elongation, and a manufacturing method therefor.

[3]

Background Art

[4] Of various materials for biomedical applications, animal-derived collagen is of high biocompatibility and histocompatibility. In addition, collagen has hemostatic activity, and is biodegradable and completely absorbed in vivo.

[5] Such collagen can be isolated and purified from connective tissues of various animal organs, including skin, bone, cartilage, tendon, etc. by acidolysis, alkalinolysis, neutral hydrolysis, or enzymolysis.

[6] Conventionally extracted collagens for biomedical materials have a molecular level of monomers or oligomers and are stored in the form of powder or liquid.

[7] Since their collagen molecules are decomposed to the degree of monomers or oligomers, conventionally extracted collagens rapidly becomes sol upon exposure to water, body fluid, or blood.

[8] To be useful as a biomedical material in the body, collagens need hardness to some degree. For this, collagens are applied to synthetic polymer materials such as nylon, silicon, etc. Additionally or alternatively, a graft made of extracted collagen is cured chemically with a crosslinking agent or physically with radiation, such as electron radiation or UV radiation, or heat in order to maintain its form for a predetermined period of time after application to the body.

[9] However, when collagen is combined with synthetic polymer materials, the synthetic polymer materials remain as foreign matter in the body after the degradation of the collagen, and thus are apt to cause side effects such as bulbils generation, inflammation, etc. Thus, synthetic polymer materials cannot be applied to any cell or organ.

[10] Though collagen materials are crosslinked, their physical properties, especially tensile strength, do not significantly improve. Hence, collagen, although subjected to a

processing treatment, is impossible to use as biomedical material requiring suturing.

[11] In addition, a crosslinking agent, such as glutaraldehyde or epoxy, when used alone, may not only exert toxicity to the body, but also degrade the collagen's intrinsic biochemical properties, especially promotive effects on cell growth.

[12] Physical crosslinking does not guarantee sufficient physical properties to the collagen graft. With physical crosslinking, the collagen is also difficult to endow with a proper absorption rate in vivo.

[13] After a surgical operation is performed on various organs including the brain to treat various diseases or injuries, the surgical site, e.g., dura mater, pericardium, pleura, peritoneum, or serosa, must or may be sealed by suturing. Because shrinkage occurs at the sutured site or the membrane is at least partially dissected, the surgical site cannot be completely sealed and the membrane is, for the most part, defected.

[14] When the defect is neglected, either the organ, such as the brain, the heart, the lung, the intestine, etc., may stick out of the defect leading to more serious complications, or the organ or its surrounding area is exposed to water or air, which disturbs the healing of surgical site.

[15] In order to overcome these problems, conventionally, lyophilized human cadaver dura mater, or an expanded polytetrafluoroethylene (EPTFE) film, a polypropylene mesh, a Teflon sheet or a Dacron sheet is used as a complement for the defect.

[16] However, these conventional artificial biomembranes, when used, for example, in human dura mater, may cause adhesion with the cerebral parenchyma, which may result in the onset of post-operative epilepsy. Particularly, EPTFE films do not degrade, but remain as foreign materials that are likely to mediate infection. Further, when tissues are in contact with the EPTFE films, cells undergo fatty degradation. As such, the conventional artificial biomembranes are prone to causing post-operative complications.

[17] Now, research has been directed toward the development of materials for biomembranes that can be sutured while retaining biochemical properties and which can maintain intended shapes for a predetermined period of time after in vivo application.

[18] With regard to related art, reference may be made to Korean Patent Unexamined Publication Application No. 10-2002-0036225 (issued on May 16, 2002, titled "Biomembrane Dressing for Healing Dermal Wound and Infection") and Korean Patent No. 10-1280722 (issued on June 25, 2013, titled "Thin film multilocular structure made of collagen, member for tissue regeneration containing the same, and method for producing the same").

[19]

Disclosure of Invention

Technical Problem

[20] It is an object of the present invention to provide a biocompatible cocoon-based biomembrane that can be relatively simply manufactured in a more cost efficient manner than conventional artificial biomembranes and which has excellent cell growth potential, and a method for manufacturing the same.

[21] It is another object of the present invention to provide a cocoon-based artificial biomembrane having excellent tensile strength and elongation, and a method for manufacturing the same.

[22] Embodiments of the present invention will be described in detail with reference to the accompanying drawings. These embodiments will be described in detail in order to allow those skilled in the art to practice the present invention. It should be appreciated that various embodiments of the present invention are different, but are not necessarily exclusive. For example, specific shapes, configurations, and characteristics described in an embodiment of the present invention may be implemented in another embodiment without departing from the spirit and the scope of the present invention. In addition, it should be understood that positions and arrangements of individual components in each disclosed embodiment may be changed without departing from the spirit and the scope of the present invention. Therefore, the detailed description provided below should not be construed as being restrictive. In addition, the scope of the present invention is defined only by the accompanying claims and their equivalents if appropriate.

[23]

Solution to Problem

[24] In order to accomplish the above objects, an aspect of the present invention provides a cocoon-based artificial biomembrane, which is prepared by dividing a cocoon having a first shell thickness into two or more fragments in a predetermined form.

[25] In one exemplary embodiment of the present invention, each of the fragments is delaminated into a lamellar fragment with a second thickness, the second thickness being smaller than the first thickness.

[26] In another exemplary embodiment of the present invention, the lamellar fragment with a second thickness is an inner, mid or outer stratum of the cocoon.

[27] In another exemplary embodiment of the present invention, the lamellar fragment is sterilized or packed.

[28] In accordance with another aspect thereof, the present invention provides a method for manufacturing a cocoon-based artificial biomembrane, comprising a first step of dividing a cocoon into two or more fragments in a predetermined form, the cocoon

having a shell with a first thickness.

[29] In one exemplary embodiment of the present invention, the method may further comprise a second step of delaminating each of the fragments into a lamellar fragment with a second thickness, the second thickness being smaller than the first thickness.

[30] In another exemplary embodiment of the present invention, the method may further comprise a third step of packing the fragments of the second thickness prepared in the second step.

[31] In another exemplary embodiment of the present invention, the method may further comprise conducting sterilization before or after each step.

[32] In another exemplary embodiment of the present invention, the lamellar fragment with a second thickness is an inner, mid or outer stratum of the cocoon.

[33]

Advantageous Effects of Invention

[34] The artificial biomembrane of the present invention is biocompatible not only because it has high tensile strength and elongation, which are necessary for biomembranes, but also because it exhibits excellent cell growth potential. In addition, the artificial biomembrane can be prepared easily and thus in a cost-efficient manner, compared to conventional artificial biomembranes.

[35]

Brief Description of Drawings

[36] FIG. 1 is a schematic view illustrating a manufacturing procedure of a cocoon-based artificial biomembrane according to one embodiment of the present invention;

[37] FIG. 2 is a schematic view illustrating the manufacturing procedure of a cocoon-based artificial biomembrane according to a modified embodiment of the present invention;

[38] FIG. 3 shows morphologies of cocoon fragments used in the artificial biomembranes of the present invention;

[39] FIG. 4 is a graph showing a mechanical property (tensile strength) of the cocoon-based artificial biomembrane of the present invention; and

[40] FIG. 5 is a graph showing the cell growth potentials of the cocoon-based artificial biomembranes of the present invention.

Best Mode for Carrying out the Invention

[41] Unless otherwise specified, the terms and techniques used in the specification should be defined to have the same meanings as are generally accepted in the art to which the present invention pertains.

[42] Unless otherwise defined, all terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which example embodiments belong. It

will be further understood that terms, e.g., those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the relevant art and will not be interpreted in an idealized or overly formal sense unless expressly so defined herein.

[43] The term "biomembrane" or "biological membrane", as used herein, refers to an enclosing or separating membrane within living things, such as cell membranes, and organelle membranes. That is, serving as a selectively permeable barrier to an external environment, a biomembrane functions to prevent the external release of nucleic acids, proteins and other biomolecules and to construct an independent space where various biological activities happen. Further, a cell membrane protects the cell from external factors, and plays as a passage for material transport between the cytoplasm and the external environment.

[44] For use as an alternative to a biomembrane, an artificial biomembrane has to maintain the intended shape thereof for a predetermined period of time after application to the body. Importantly, the artificial biomembrane must avoid adhesion with tissues around the surgical site after surgical operation, mediation of infection, and tissue degeneration, and must promote regeneration.

[45] Artificial biomembranes find applications in various fields including artificial neural tubes, artificial cord cervical spines, artificial esophagus, artificial bronchi, artificial vessels, artificial valves, artificial dura mater, and artificial ear drums.

[46] For obtaining these properties, artificial biomembranes used thus far need pretreatment processes, such as physical crosslinking, etc. However, such pretreated artificial biomembranes may be toxic to the body and remain as foreign matter upon long-term use in vivo. Moreover, another disadvantage is that the pretreatment processes and chemicals used therefor increases the production cost of the artificial biomembranes.

[47]

[48] Leading to the present invention, intensive and thorough research into an artificial biomembrane, conducted by the present inventor, resulted in the finding that a fragment prepared from a cocoon is biocompatible and useful as a biomembrane not only because it has high tensile strength and elongation but also it exhibits an excellent cell growth potential, and that the fragment can be produced at significantly lower cost, compared to conventional artificial biomembranes.

[49] With reference to FIG. 1, a method for manufacturing a cocoon-based artificial biomembrane in accordance with the present invention is explained, below.

[50] 1. Step 1: Preparation of cocoon fragment with first thickness.

[51] As shown in FIG. 1A, a cocoon 10, the shell of which has a first thickness, is prepared.

[52] A cocoon is a casting spun of silk by silkworms and is used as a material of silk fibers. In the present invention, cocoons, which may be idle resources, are up-cycled into a new high added-value product, thus bringing economic benefits to silkworm farmers.

[53] Naturally constructed by silkworms, which eat clean mulberry leaves, cocoons are free of toxicity and are suitable for use as an environment-friendly material.

[54] Hence, the present invention takes a cocoon 10 as a material for an artificial biomembrane. The cocoon 10 is processed, as shown in FIGS. 1B to 1E, into two or more planar fragments, each having a first thickness.

[55] In greater detail, the oval cocoon 10 is dissected along a cutting line 11 into halves, as shown in FIG. 1B. The dissected halves have semi-oval shapes, and are opened to expose the inside surface 13 of the cocoon, as shown in FIG. 1C.

[56] Next, the cocoon halves with curved inside surfaces 13 are planarized to some degree by cutting many sites along the edge as shown in FIG. 1D, and planar regions are cut out to obtain cocoon fragments with a first thickness, as shown in FIG. 1E.

[57] The artificial biomembrane prepared in the present invention needs not have a planar surface. Because a cocoon originally has an elliptical ball shape, the curved shape of the dissected cocoon may be utilized to give curved artificial biomembranes if necessary. For use as a small artificial biomembrane, a cocoon fragment with a small area may be relatively planar. In contrast, when taken a relatively large area of the dissected cocoon halves, the artificial biomembranes may have curved surfaces.

[58] With reference to FIG. 2, a modified method for preparing a cocoon fragment with a first thickness is described. In detail, an oval-shaped cocoon 10 is cut along cutting lines 15 and 17 to expose the inside 13 of the cocoon 10, as shown in FIGS. 2B and 2C. Then, the dissected cocoon with a curved surface is spread as shown in FIG. 2D, and then cocoon fragments 20 with a first thickness are obtained as shown in FIG. 2E.

[59] The preparation methods of cocoon fragments described in FIGS. 1 and 2 are only illustrative, but are not intended to limit the manufacturing method of artificial biomembranes according to the present invention. A cocoon fragment with a first thickness may be prepared by cutting a cocoon in the manners shown in FIGS. 1 and 2, but other cutting methods may be used.

[60] 2. Step 2: Preparation of cocoon fragment with second thickness (artificial biomembrane)

[61] Because the cocoon fragments 20 with a first thickness, prepared in step 1, has a multilayer structure identical to that of the cocoon shell, the multilayer structure may be split into thinner layers for use as an artificial biomembrane.

[62] Although the cocoon fragment 20 with a first thickness, prepared in step 1, is itself possible to be used as an artificial biomembrane, it is subjected to thickness splitting to

give cocoon fragments 30 with a second thickness. In this regard, the second thickness is smaller than the first thickness.

[63] As they are, the cocoon fragments 30 can be applied for any purpose of artificial biomembranes. If necessary, they may be sterilized or chemically treated.

[64] In detail, a cocoon shell varies in thickness (first thickness) from 0.3 to 1.0 mm depending on silkworm species.

[65] In principal, any kind of cocoons may be used in the present invention. For the purpose of the present invention, a cocoon with a shell thickness of 0.5~0.8 mm is employed. In the present invention, the cocoon shell is divided into inner, mid and outer strata. First, the outer stratum is defined as a layer that is deep from the outer surface to a point corresponding to 25 % of the total shell thickness. The inner stratum is defined as a layer that is deep from the inner surface to a point corresponding to 15 % of the total shell thickness. The other part corresponding to 60 % of the total shell thickness, that is, the remaining middle cocoon shell except the outer and the inner layer is the mid stratum. The numerical values of the interlayer borders are determined according to characteristics of individual strata (inner, mid, and outer), as shown in FIG. 3. That is, the outer stratum that is deep from the outer surface to a point corresponding to 25 % of the total shell thickness can be used as a cocoon fragment characterized by high elongation. The mid stratum that has a thickness corresponding to 60 % of the total shell thickness exhibits high cell growth potential thanks to its high porosity. The inner stratum that accounts for the remaining 15 % of the total shell thickness has a smooth surface and high tensile strength.

[66] A cocoon fragment can be easily delaminated into up to 16 lamellas although the number of delaminations is dependent on the shell thickness. The thicknesses of the lamellas can be determined according to strength and elongation necessary for the kind and use of the artificial membrane. From a cocoon with a shell thickness of 0.5~0.8 mm, an artificial biomembrane 0.01mm ~ 0.7mm thick can be prepared by delamination. According to the use of the artificial biomembrane, selection may be made of the cocoon fragments 30 with various thicknesses.

[67] As can be seen FIG. 4, the outer stratum 35 is suitable as an elastic biomembrane because it is higher in elongation rate than the inner stratum 31 and the mid stratum 33. The highest elongation rate is measured from the outer stratum, with the lowest elongation rate from the inner stratum.

[68] The inner stratum 31 has a smooth surface so that it is unlikely to adhere. In addition, the inner stratum has high tensile strength so that it is suitable for use as a biomembrane where strength is needed. The highest tensile strength is measured in the inner stratum, with the lowest tensile strength in the outer stratum.

[69] Further, the mid stratum 33 exhibits has excellent cell growth potential because of its

high porosity. Hence, it is suitably applied as a biomembrane to a defect lesion that needs fast healing.

Mode for the Invention

[70] A better understanding of the present invention may be obtained through the following examples that are set forth to illustrate, but are not to be construed as limiting the present invention.

[71]

[72] <EXAMPLE 1> Preparation of Artificial Biomembrane 1

[73] A cocoon 10 was prepared, and cut at a proper site to exposure the inside thereof.

[74] Next, the cut cocoon was further processed to make the curved inside planar.

[75] The planarized cocoon was cut into rectangular fragments 20.

[76] From the cocoon fragments 20, a layer containing the innermost surface 13, that is, an inner stratum was delaiminated at a thickness of 0.07 mm.

[77] The delaminated inner stratum was sterilized and used as an artificial biomembrane 1.

[78]

[79] <EXAMPLE 2> Preparation of Artificial Biomembrane 2

[80] From the remaining cocoon after the inner stratum was delaminated in Example 1, a layer containing the outermost surface, that is, an outer stratum opposite to the inner stratum was delaminated at a thickness of 0.07 mm.

[81] The delaminated outer stratum was sterilized and used as an artificial biomembrane 2.

[82]

[83] <EXAMPLE 3> Preparation of Artificial Biomembrane 3

[84] The remainder after the inner stratum and outer stratum were delaminated into mid strata, each 0.07 mm thick, followed by sterilization to give an artificial biomembrane 3.

[85]

[86] <TEST EXAMPLE 1> Morphology of Artificial Biomembranes from Cocoon

[87] 1. Test Method

[88] Morphologies of the artificial biomembranes prepared in Examples 1 to 3 were observed by scanning electron microscopy (SEM) and with the naked eye. The results are shown in FIG. 3.

[89] 2. Test Results

[90] As can be seen in FIG. 3, the inner stratum (A, Example 1), the mid stratum (B, Example 3), and the outer stratum (C, Example 2) were different from one another in terms of morphological properties, such as fiber thickness, pore form, etc. Under the

naked eye, the mid stratum was observed to have a smoother surface. Thus, the morphological results indicate that the inner stratum, the mid stratum, and the outer stratum can be used where their unique characteristics are needed.

[91] For example, as shown in FIG. 3, the inner stratum has a smooth surface and is water resistant so that it is suitably used as an artificial biomembrane where a non-sticky property is needed. Having high cell growth potential thanks to the high porosity thereof, the mid stratum can be suitably used as a biomembrane in a defect region that needs fast healing.

[92]

[93] <TEST EXAMPLE 2> Physical Properties of Cocoon-Based Artificial Biomembrane According to Cocoon Stratum

[94] 1. Test Method

[95] Physical properties of the cocoon-based artificial biomembranes prepared in Examples 1 to 3 by cocoon stratum were measured. In this regard, a tensile test was conducted using a universal testing machine (DAEYEONG, Korea).

[96] Test specimens with sizes of 2.5 x 0.07 (width x length) mm were used. The specimens were extended at a rate of 10 mm/min, with an initial gauge length set to be 10 mm.

[97] Results are given in FIG. 4 (strain (mm) versus stress (MPa)) and Table 1, below.

[98] 2. Test Results

[99]

[100] Table 1

[Table 1]

	Tensile Strength (MPa)	Elongation (%)
Inner Stratum (Ex. 1)	60.20±5.3	12.45±1.5
Mid Stratum (Ex. 3)	46.19±2.2	15.05±1.7
Outer Stratum (Ex. 2)	29.36±3.1	18.93±1.3

[101]

[102] As is understood from the data of FIG. 4 and Table 1, the cocoon-based artificial biomembranes were different from one another in tensile strength and elongation by stratum. The highest tensile strength was detected in the inner stratum while the highest elongation was measured from the outer stratum.

[103] In other words, the inner stratum is suitable for use as an artificial biomembrane in a site where strength is important whereas the outer stratum is preferably applied to a site that needs elasticity.

[104]

[105] <TEST EXAMPLE 3> Physical Properties of Mid Cocoon Stratum-Based Artificial Biomembrane According to Thickness

[106] 1. Test Method

[107] Physical properties of the mid stratum-based artificial biomembranes were measured according to thickness. In this regard, a tensile test was conducted using a universal testing machine (DAEYEONG, Korea). Test specimens with sizes of 20 x 2.5 (width x length) mm were used in a dry state or a wet state. In the latter case, the specimens were immersed in physiological saline for 1 hrs. For a control, a commercially available collagen membrane was employed. The specimens were extended at a rate of 10 mm/min, with an initial gauge length set to be 10 mm. Results are given in Table 2, below.

[108]

[109] 2. Test Results

[110] Table 2

[Table 2]

Kind	Thick.(mm)	Max. Load (N)		Tensile Strength (MPa)		Elongation (%)	
		Dry	Wet	Dry	Wet	Dry	Wet
Mid Stratum	0.01	6	3	2	13	2	23
	0.02	9	8	2	20	1.4	29.4
	0.04	17	11	4	14	1.8	23
	0.06	16	16	4	13	1.6	31.8
	0.08	33	15	8	9	2.4	25.2
	0.1	37	20	9	10	2	30.2
	0.2	88	62	22	16	2.8	26.6
	0.4	113	80	28	10	2.8	31.6
Collagen	0.2	3.3	0.25	0.8	0.05	7.8	18.8

[111]

[112] As is understood from the data of Table 2, the cocoon-based artificial biomembrane differs in tensile strength and elongation by thickness. Both the tensile strength and the elongation increased with the increase of thickness, and were better in a wet state than in a dry state. In contrast, the tensile strength of the commercially available collagen membrane decreased 16 times when it was in a wet state compared to when it was in a

dry state. Taken together, the data indicates that physical properties of the cocoon-based biomembranes can be maintained for a longer period of time than those of the wet collagen membrane.

[113]

[114] <TEST EXAMPLE 4> Cell Growth Potential of Artificial Biomembrane

[115] 1. Test Method

[116] In order to test the artificial biomembranes for cell growth potential, the mouse fibroblast cell line L929 was cultured on the artificial biomembranes at 37°C in a 5% CO_2 condition. For this, DMEM (Dulbecco's modified Eagles medium-high glucose, WelGENE, Korea) supplemented with 10% (v/v) FBS (fetal bovine serum, GIBCO), and 100 U streptomycin and 100 μg/ml penicillin (GIBCO) was used. The artificial biomembrane was assayed for cell growth potential by MTT.

[117] The results are depicted in FIG. 5 wherein cell growth potential (relative activity) is given according to cocoon stratum.

[118]

[119] 2. Test Result

[120] As can be seen in FIG. 5, all the cocoon-based artificial biomembranes exhibited good cell growth potential, with 4-fold higher cell growth on the mid stratum than the inner stratum.

[121] In other words, the mid stratum is more suitable than the inner stratum for use in a site that needs cell growth. In addition, the cocoon-based artificial biomembranes of the present invention were observed to grow the cells at higher efficiency, compared to the collagen membrane.

[122] 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.

[123]

[124] <Description of Numerical References in Drawings>

[125] 10: Cocoon

[126] 11: cutting line 1

[127] 13: Inside surface

[128] 15: cutting line 2

[129] 17: cutting line 3

[130] 20: Cocoon fragment with a first thickness

[131] 30: Cocoon fragment with a second thickness

[132] 31: Inner stratum

[133] 33: Mid stratum

[134] 35: Outer stratum

Claims

[Claim 1] A cocoon-based artificial biomembrane, prepared by dividing a cocoon into two or more fragments in a predetermined form, the cocoon having a shell with a first thickness.

[Claim 2] The cocoon-based artificial biomembrane of claim 1, wherein each of the fragments is delaminated into a lamellar fragment with a second thickness, the second thickness being smaller than the first thickness.

[Claim 3] The cocoon-based artificial biomembrane of claim 2, wherein the lamellar fragment with a second thickness is an inner stratum of the cocoon.

[Claim 4] The cocoon-based artificial biomembrane of claim 2, wherein the lamellar fragment with a second thickness is a mid stratum of the cocoon.

[Claim 5] The cocoon-based artificial biomembrane of claim 2, wherein the lamellar fragment with a second thickness is an outer stratum of the cocoon.

[Claim 6] The cocoon-based artificial biomembrane of claim 3, wherein the lamellar fragment is sterilized.

[Claim 7] The cocoon-based artificial biomembrane of claim 4, wherein the lamellar fragment is sterilized.

[Claim 8] The cocoon-based artificial biomembrane of claim 5, wherein the lamellar fragment is sterilized.

[Claim 9] The cocoon-based artificial biomembrane of any one of claims 6 to 8, wherein the lamellar fragment is packed.

[Claim 10] A method for manufacturing a cocoon-based artificial biomembrane, comprising a first step of dividing a cocoon into two or more fragments in a predetermined form, the cocoon having a shell with a first thickness.

[Claim 11] The method of claim 10, further comprising a second step of de-laminating each of the fragments into a lamellar fragment with a second thickness, the second thickness being less than the first thickness.

[Claim 12] The method of claim 10 or 11, further comprising a third step of packing the fragments of the second thickness prepared in the second step.

[Claim 13] The method of claim 10 or 11, further comprising conducting sterilization before or after each step.

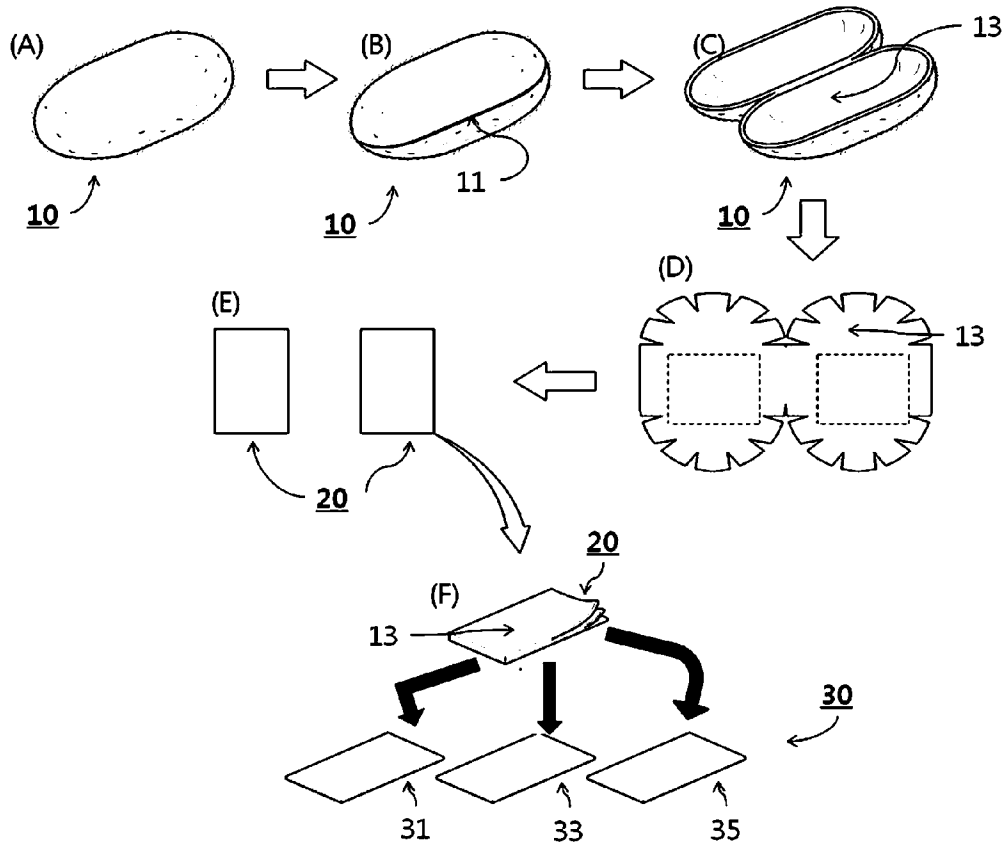
[Claim 14] The method of claim 12, wherein the lamellar fragment with a second

thickness is an inner stratum of the cocoon.

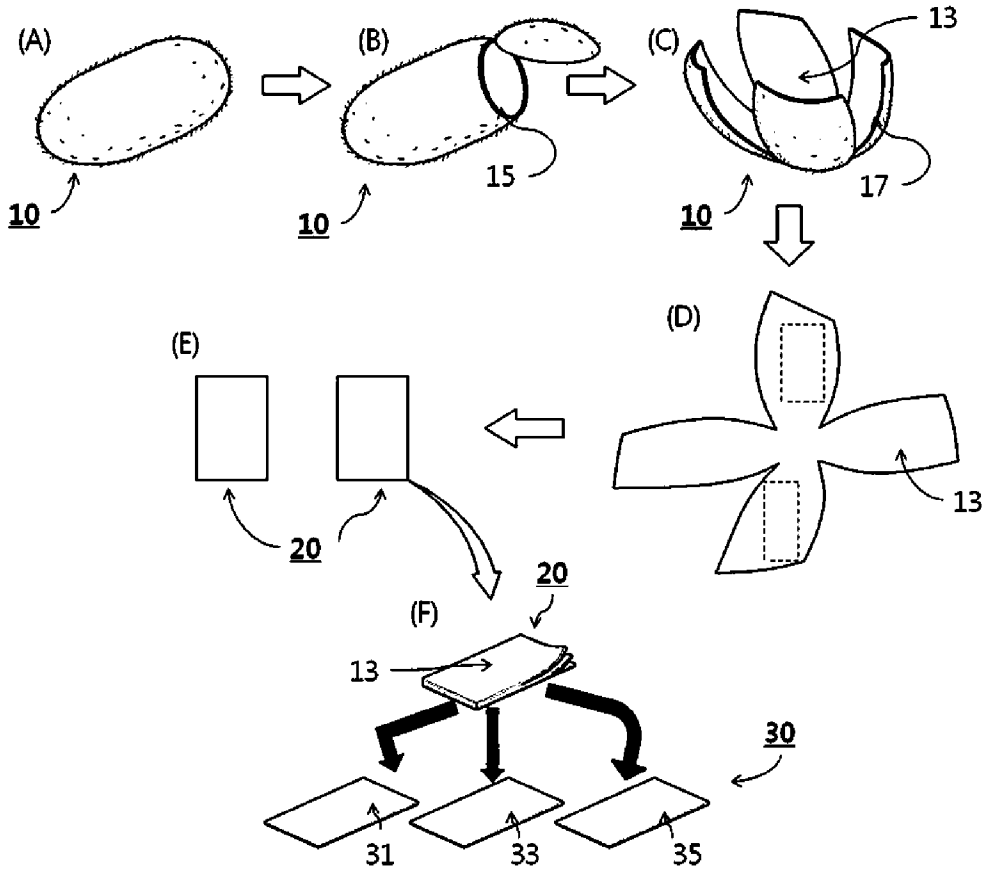
[Claim 15] The method of claim 12, wherein the lamellar fragment with a second thickness is a mid stratum of the cocoon.

[Claim 16] The method of claim 12, wherein the lamellar fragment with a second thickness is an outer stratum of the cocoon.

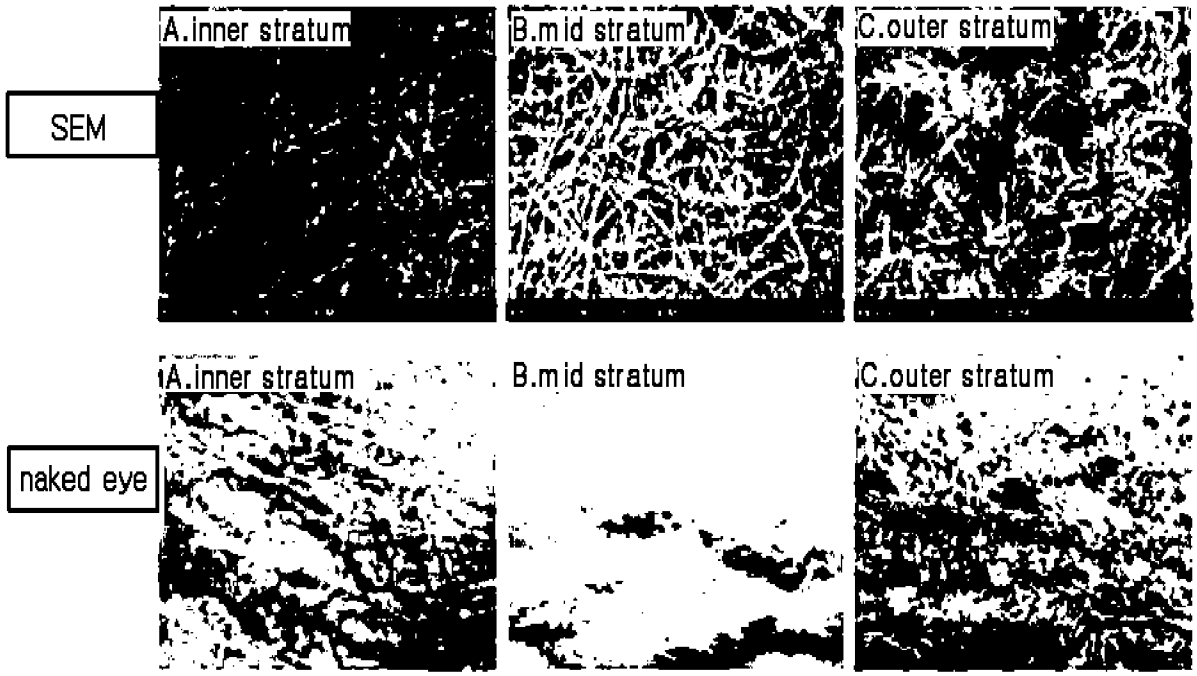
[Fig. 1]


(A)
(B)
(C)
13
10
11
10
10
(D)
(E)
13
20
20
(F)
13
30
31
33
35

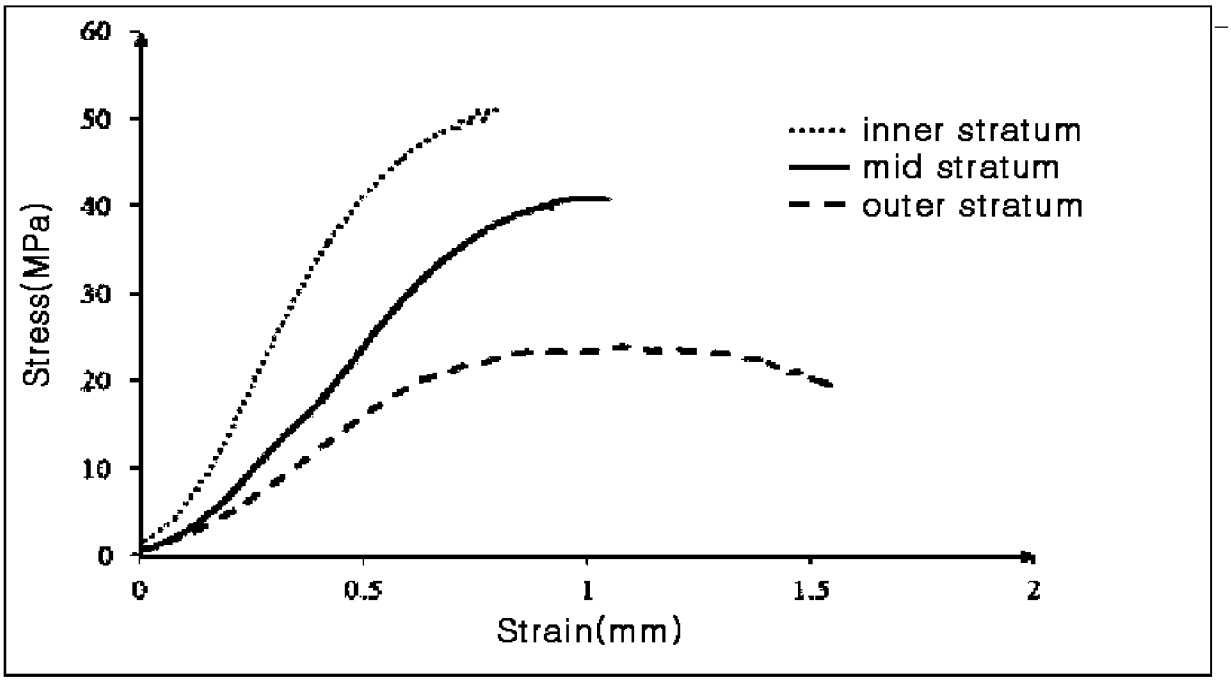
[Fig. 2]


(A)
(B)
(C)
13
10
15
10
17
10
(D)
(E)
13
20
20
(F)
13
30
31
33
35

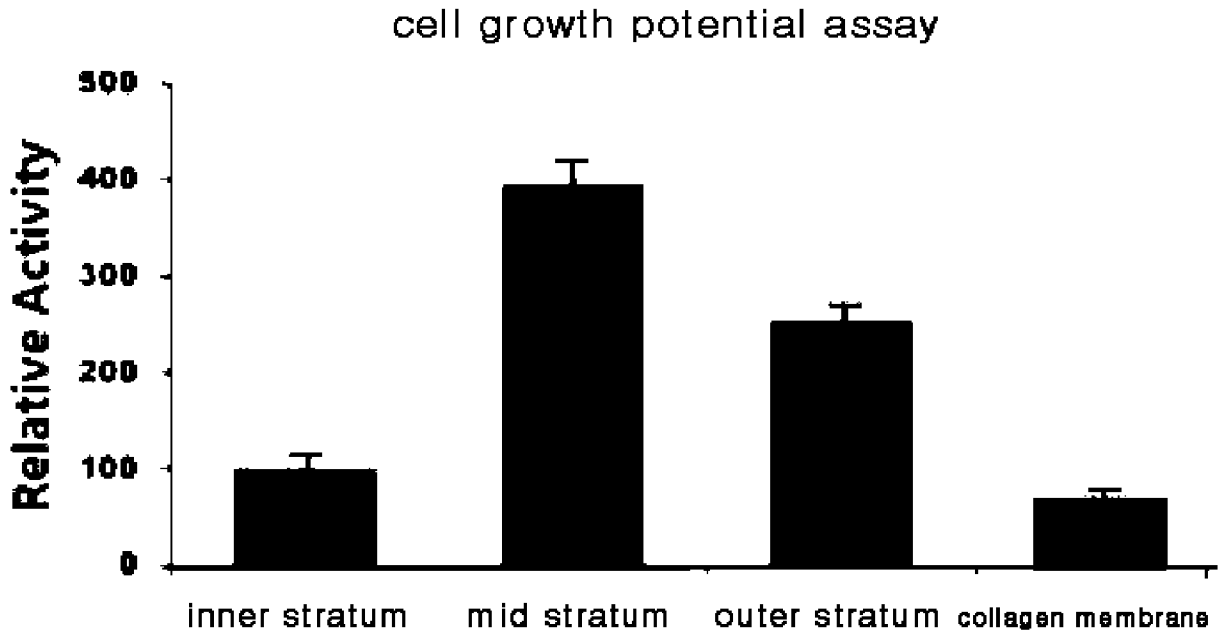
[Fig. 3]


A.inner stratum
B.mid stratum
C.outer stratum
SEM
A.inner stratum
B.mid stratum
C.outer stratum
naked eye

[Fig. 4]


60
50
40
30
20
10
0
Stress(MPa)
0
0.5
1
1.5
2
Strain(mm)
inner stratum
mid stratum
outer stratum

[Fig. 5]


cell growth potential assay
Relative Activity
500
400
300
200
100
0
inner stratum
mid stratum
outer stratum
collagen membrane

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2015/005925

A. CLASSIFICATION OF SUBJECT MATTER

A61L 27/22(2006.01)i, A61F 2/02(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L 27/22; C08L 1/24; C08L 5/08; A61F 2/02; D01F 4/02; C09F 1/00; A61K 38/00; A61F 2/18; B82Y 30/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: cocoon, artificial, biomembrane, peel off, shell, thickness, delaminate, lamellar, fragment, stratum, sterilize

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 06-166850 A (MURASE MARIE) 14 June 1994 See abstract; claims 1-5,8,9; paragraphs [0010].	10,11,13
A		1-9,12,14-16
A	JO, Y. Y. et al., J. SERIC. ENTOMOL. SCI. 51(1), pp.68-72 (2013) See the entire document.	1-16
A	KR 10-2010-0121169 A (HALLYM UNIV. INDUSTRY FOUNDATION et al.) 17 November 2010 See abstract; claims 1-11.	1-16
A	US 2004-0097709 A1 (UBALDO ARMATO et al.) 20 May 2004 See abstract; claims 1-16.	1-16
A	KR 10-2006-0038096 A (SEOUL NATIONAL UNIV. INDUSTRY FOUNDATION) 03 May 2006 See abstract; claims 1-10.	1-16
A	KR 10-2003-0097691 A (NATIONAL INSTITUTE OF AGROBIOLOGICAL SCIENCES) 31 December 2003 See abstract; claims 1-14.	1-16

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"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	Date of mailing of the international search report
05 October 2015 (05.10.2015)	**06 October 2015 (06.10.2015)**

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2015/005925

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