

DESCRIPTION

Invention Title

LOW-EXTRUSION COMPOSITION COMPRISING ACELLULAR DERMAL
MATRIX AND METHOD FOR PREPARING SAME

5

Technical Field

[0001] The present disclosure relates to a low-extrusion composition including an acellular dermal matrix (ADM) and a method for preparing the same.

10 Background Art

[0002] Acellular dermal matrix is one of injectable biological tissue substitutes and used for wound healing as well as soft tissue reinforcement by promoting the regeneration of existing tissue to repair defects. Such an
15 acellular dermal matrix is applied for wound treatment in various forms, including injectables, dressings, adhesives, surgical and medical devices, artificial skin, bandages, foaming agents, films, anti-adsorption agents, and implants.

[0003] Meanwhile, when acellular dermal matrix is used in
20 injectable form, there have been problems such as high extrusion force that is difficult for the operator to control, increased patient pain, and the risk of instantaneous shock. Therefore, although attempts have been made to reduce extrusion force by adding lubricants such as hyaluronic acid,

the use of these lubricants has been limited due to the presence of these components, preventing their diverse applications.

[0004] Therefore, there is an urgent need to develop a
5 component that can reduce extrusion force while replacing existing lubricants.

DISCLOSURE

Technical Problem

[0005] An object of the present disclosure that has been
10 derived to solve the above-described problems is to provide a low-extrusion composition including an acellular dermal matrix with reduced extrusion force characteristics compared to the existing one by applying maltitol, and a method for preparing the same.

15 Technical Solution

[0006] To achieve the above objective, a low-extrusion composition including an acellular dermal matrix according to one embodiment of the present disclosure includes 100 parts by weight of an acellular dermal matrix, 100-3,000
20 parts by weight of water, and 1-1,000 parts by weight of maltitol.

[0007] A method for preparing a low-extrusion composition including an acellular dermal matrix according to another embodiment of the present disclosure includes the steps of:

(a) preparing a human-derived acellular dermal matrix that has been deepidermized, defatted, and decellularized; (b) adding a maltitol solution to the acellular dermal matrix to infiltrate it; (c) freeze-drying the infiltration-completed
5 acellular dermal matrix and then pulverizing it; and (d) adding water to the pulverized acellular dermal matrix to hydrate and paste it.

Advantageous Effects

[0008] According to one embodiment of the present disclosure,
10 a low-extrusion composition including an acellular dermal matrix can improve the convenience and safety of the procedure by lowering the average extrusion force by applying maltitol in an amount of 1-1,000 parts by weight.

Brief Description of Drawings

15 [0009] FIGS. 1 and 2 are process diagrams for explaining a method for preparing a low-extrusion composition including an acellular dermal matrix according to another embodiment of the present disclosure.

[0010] FIG. 3 is a graph showing results according to
20 Experimental Example 1.

[0011] FIGS. 4 to 6 are graphs showing results according to Experimental Example 2.

[0012] FIG. 7 is a photograph showing a mixture of ADM and

purified water, and FIG. 8 is a graph showing results according to Experimental Example 3.

Mode for Invention

[0013] Hereinafter, the present disclosure will be described
5 in detail as exemplary embodiments thereof with reference to
the attached drawings. However, the following exemplary
embodiments are provided as illustrative examples of the
present disclosure, and if a detailed description of a
technology or configuration well known to those skilled in
10 the art is deemed to unnecessarily obscure the gist of the
present disclosure, such detailed description may be omitted,
and the present disclosure is not limited thereby. The
present disclosure is capable of various modifications and
applications within the scope of the description of claims
15 described later and equivalents interpreted therefrom.

[0014] Furthermore, the terminologies used in the present
specification are terms used to appropriately express
preferred embodiments of the present disclosure, and these
may vary depending on the intent of the user or operator or
20 the practices of the field to which the present disclosure
pertains. Therefore, definitions of the present terms should
be based on the overall content of the present specification.
Throughout the specification, when a part is said to
"includes" a component, it may mean that this does not

exclude other components, but rather include other components, unless otherwise stated.

[0015] Throughout the present specification, in the term "%" that is used to indicate the concentration of a specific substance, "%" refers to solid/solid (w/w), "%" refers to solid/liquid (w/v), and "%" refers to liquid/liquid (v/v), unless otherwise specified.

[0016]

[0017] Hereinafter, a low-extrusion composition including an acellular dermal matrix according to one embodiment of the present disclosure will be described in detail.

[0018] The low-extrusion composition including an acellular dermal matrix of this embodiment includes an acellular dermal matrix, water, and maltitol. In this embodiment, the extrusion force, which was relatively high at 100 N or more, was reduced to 20 N or less by applying maltitol, which acts as a lubricant in present disclosure, thereby improving the convenience and safety of the procedure. That is, although individual differences may occur, the present disclosure was able to achieve an extrusion force of 20 N or less, which is generally a level that allows for injection without clogging.

[0019] Acellular dermal matrix (ADM) is a component widely used in plastic surgery and orthopedics for the purpose of

reconstruction, regeneration, and reinforcement of skin, tendons, and ligaments due to its superior biocompatibility resulting from high cell adhesion ability and low immune response compared to existing animal-derived products. This
5 acellular dermal matrix may be contained in an amount of 100 parts by weight.

[0020] Water is an ingredient added for hydration and may be contained in an amount of 100-3,000 parts by weight per 100 parts by weight of acellular dermal matrix. If the amount of
10 water is less than 100 parts by weight, it may be difficult to hydrate the acellular dermal matrix and maltitol, and if it exceeds 3,000 parts by weight, it may be difficult to achieve the desired formulation due to excessive use.

[0021] Maltitol prevents a reduction in extrusion force
15 within the syringe and a browning phenomenon of the tissue as an ingredient that acts as a lubricant, and may be contained in an amount of 1-1,000 parts by weight per 100 parts by weight of acellular dermal matrix. If the amount of maltitol is less than 1 part by weight, the effect on the
20 reduction in extrusion force may be negligible, and if it exceeds 1,000 parts by weight, the effect of reducing extrusion force due to excessive addition may not be very significant.

[0022] In this embodiment, one or more additives selected from

a hypotonic solution, an isotonic solution, a buffer solution, a surfactant, glycerol, and propylene glycol may be further included, and since it is desirable for the additive to be managed at 50 ppm or less as a
5 decellularization reagent, it may be contained in an amount of 0.00001-0.00125 parts by weight per 100 parts by weight of acellular dermal matrix.

[0023] In this embodiment, it goes without saying that the low-extrusion composition including the acellular dermal
10 matrix can be used as an injectable, a dressing, an adhesive, a surgical or medical device, an artificial skin, a bandage, a foaming agent, a film, an anti-adsorption agent, or an implant.

[0024] Meanwhile, when the low-extrusion composition
15 including the acellular dermal matrix of the present disclosure is injected into the body, since it acts as a scaffold, thereby activating cells and growth factors within the body to aid in the regeneration, recovery, and strengthening of soft tissues, it can be used for the purpose
20 of recovery and regeneration of defective or damaged muscles, biological membranes, ligaments, and tendons during internal and external medical treatments and surgeries. Here, soft tissue refers to muscles, ligaments, fat, blood vessels, nerves, tendons, fibrous tissue, synovial tissue, etc., and

collectively refers to the rest of the body's connective tissues excluding hard tissues, namely cartilage and bone, as well as blood and hematopoietic tissue.

[0025]

5 **[0026]** Hereinafter, a method for preparing a low-extrusion composition including an acellular dermal matrix according to another embodiment of the present disclosure will be described in detail with reference to the drawings.

[0027] FIGS. 1 and 2 are process diagrams for explaining a
10 method for preparing a low-extrusion composition including an acellular dermal matrix according to another embodiment of the present disclosure.

[0028] Referring to FIGS. 1 and 2, first, a human-derived acellular dermal matrix that has been deepidermized,
15 defatted, and decellularized is prepared (S10).

[0029] The acellular dermal matrix can be prepared by the steps of preparing a human-derived skin tissue that has not undergone a separate deepidermization and defatting process (S11), treating the skin tissue with a hypotonic solution
20 containing a surfactant (S12), and washing the treatment-completed skin tissue with an isotonic solution (S13).

[0030] For example, the surfactant may be an ionic surfactant, preferably sodium dodecyl sulfate (SDS). The hypotonic

solution may contain one or more selected from Tris-HCl, ethylenediaminetetraacetic acid (EDTA), and sodium hydroxide (NaOH), preferably 0.1-1.5 M EDTA, and the osmolality may be 270 mOsm/L or less. The isotonic solution may be one or more
5 selected from Tris-HCl, ethylenediaminetetraacetic acid (EDTA), sodium hydroxide (NaOH), and sodium chloride (NaCl), and the osmolality may be 270-300 mOsm/L.

[0031] In the present disclosure, osmolarity refers to the expression of osmotic concentration per volume of a solution
10 (mOsm/L), and the osmolarity of plasma in the body and other body fluids is 270-300 mOsm/L.

[0032] The method may further include a step of washing the skin tissue with a buffer solution between the steps S12 and S13 and after the step S13, wherein the buffer solution may
15 be one or more selected from phosphate buffer saline (PBS), distilled water, physiological saline, Hank's balanced salt solution (HBSS), Tris buffered saline (TBS), N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), Bicine (N,N-Bis(2-hydroxyethyl) glycine), 4-(2-
20 hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), NTris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), piperazine-N,N'-bis(2-ethanesulfonic acid (PIPES), sodium cacodylate, potassium cacodylate, 2-(N-morpholino)ethanesulfonic acid (MES), minimum essential

media (MEM), Dulbecco's modified eagle media (DMEM),
RPMI1640, Iscove's modified Dulbecco's media (IMDM),
Keratinocyte-SFM (without bovine pituitary extract (BPE)),
Keratinocyte-SFM (with BPE), KnockOut DMEM, AmnioMAX-II
5 complete medium, and AmnioMAX-C100 complete medium.

[0033]

[0034] Next, a maltitol solution is added to the acellular
dermal matrix to infiltrate it (S20).

[0035] 20-40 parts by weight of the maltitol solution may be
10 added to 100 parts by weight of the acellular dermal matrix
prepared in the step S10, and infiltrated in a low-
temperature reactor at 3-5°C for 10-30 hours. If less than
20 parts by weight of the maltitol solution is added, the
content of maltitol infiltrating the acellular dermal matrix
15 is lowered so that the effect of reducing extrusion force
may be negligible, and if more than 40 parts by weight of
the maltitol solution is added, the content of maltitol
infiltrating the acellular dermal matrix increases, but the
resulting effect of reducing extrusion force may be
20 negligible. If the infiltration time is less than 10 hours,
the effect of improving the maltitol infiltration rate into
the acellular dermal matrix may be negligible, and if it
exceeds 30 hours, the effect according to the excess time
may not be very significant.

[0036] In this embodiment, the maltitol solution may be a mixed solution in which glycerol, propylene glycol, and a buffer solution are mixed, containing maltitol at a concentration of 20-40%, preferably 25-35%. The
5 aforementioned concentration of maltitol may take into account the content of maltitol infiltrating the acellular dermal matrix. The mixed solution may be a mixture of glycerol, propylene glycol, and a buffer solution at a weight ratio of 1:0.1-2:7-9. As the buffer solution has been
10 described in detail above, it will be omitted here.

[0037]

[0038] Then, the infiltration-completed acellular dermal matrix is freeze-dried and then pulverized (S30).

[0039] The infiltration-completed acellular dermal matrix may
15 be pulverized into a fibrous form using one or more pulverizers selected from a cutting mill, a food processor, a Mano pulverizer, and a freezer mill so that the proportion in which the ratio of the short axis to the long axis is 1:1-2,000 is 70% or more. In this embodiment, the long axis
20 length may be in the range of 10-2,000 μm . If the range deviates from the ratio of the short axis to the long axis or the long axis length mentioned above, it may be difficult to achieve the viscosity and flowability required in this embodiment when performing paste-forming as described below.

[0040]

[0041] Finally, water is added to the pulverized acellular dermal matrix to hydrate and paste it (S40).

[0042] 100-2,500 parts by weight of water may be added to 100
5 parts by weight of the acellular dermal matrix pulverized in
the step S30 to hydrate and paste it. If the water content
is less than 100 parts by weight, it may be difficult to
hydrate the acellular dermal matrix infiltrated with
maltitol, and if it exceeds 2,500 parts by weight, it may be
10 difficult to achieve the desired formulation due to excessive
use.

[0043]

[0044] After the step S40, a step (S50) of sterilizing the
paste-formed acellular dermal matrix by irradiating it with
15 radiation (E-beam) at a dose in the range of 11-30 kGy may
be further included.

[0045] After filling the paste-formed acellular dermal matrix
from the step S40 into a syringe, sterilization may be
performed by irradiating it with radiation (E-beam) at a dose
20 in the range of 11-30 kGy. However, if the dose exceeds 30
kGy, the tissue may undergo degeneration and structural
destruction due to the relatively high dose in the case of
human tissue. In this embodiment, radiation may be irradiated

at the aforementioned dose range, thereby killing only the bacteria while minimizing the destruction and degeneration of human tissue.

[0046]

5 **[0047]** Hereinafter, the present disclosure will be further described using Examples. It will be apparent to those skilled in the art that these Examples are intended solely to describe the present disclosure more specifically and that the scope of the present disclosure is not limited thereby.

10 **[0048]**

[0049] Preparation Example 1. Preparation of Acellular Dermal Matrix

[0050] Skin tissue was purchased from EURO skin bank, Allosource, CTS, tissues with a thickness of 1 mm or more
15 were selected, adipose tissue attached to the skin tissue was removed using forceps, and then the tissue was washed three times with sterile water. Next, the tissue was immersed in a hypotonic solution (Tris-HCl, EDTA, NaOH, and SDS) and treated for 6 hours. The skin tissue was then washed with
20 PBS at 4°C to remove fat, epidermis, cells, and hypotonic solution remaining in the skin tissue, and the tissue was washed with an isotonic solution (Tris-HCl, EDTA, NaCl, and NaOH) for 6 hours after overnight incubation to prepare a

deepidermized, defatted, and decellularized human-derived acellular dermal matrix.

[0051]

[0052] Preparation Examples 2 to 7. Preparation of Maltitol Solution

[0053] A maltitol solution in which glycerol (Sigma, USA), glycol (Sigma, USA), and phosphate buffer solution (Gibco, USA) were mixed at a weight ratio of 1:1:8 was prepared such that the maltitol concentration was within the range of Table 1 below.

[0054] [Table 1]

Classification	Maltitol concentration (%)
Preparation Example 2	0
Preparation Example 3	7.15
Preparation Example 4	14.3
Preparation Example 5	17.9
Preparation Example 6	21.45

Preparation	25
Example 7	

[0055]

[0056] Examples 1 to 16. Preparation of Low-Extrusion Compositions

[0057] A maltitol solution was added to the acellular dermal matrix prepared in Preparation Example 1 and infiltrated in a low-temperature reactor at 4°C. Then, the mixture was placed in a Tyvek (Korea Advanced Materials, Korea) and freeze-dried for 24 hours in a freeze-dryer with a vacuum of 5 torr, after which it was pulverized using a cutting mill (Pulverisette 19, FRITSCH, Germany). Next, 3cc of distilled water was added to 1g of the pulverized acellular dermal matrix and mixed with a stirrer at room temperature. The mixture was placed in a syringe, double-packaged in a PET tray and Tyvek, and then irradiated with radiation (E-beam) at a dose of 11-30 kGy and sterilized to prepare a low-extrusion composition (hereinafter referred to as 'ADM composition or ADM').

[0058] [Table 2]

Classification	Maltitol content	Maltitol infiltration time	Whether sterilization had been

			performed or not
Example 1	Preparation Example 2	12	0
Example 2	Preparation Example 3	12	0
Example 3	Preparation Example 4	12	0
Example 4	Preparation Example 6	12	0
Example 5	Preparation Example 2	24	X
Example 6	Preparation Example 3	24	X
Example 7	Preparation Example 4	24	X
Example 8	Preparation Example 5	24	X
Example 9	Preparation Example 6	24	X
Example 10	Preparation Example 7	24	X

Example 11	Preparation Example 2	24	0
Example 12	Preparation Example 3	24	0
Example 13	Preparation Example 4	24	0
Example 14	Preparation Example 5	24	0
Example 15	Preparation Example 6	24	0
Example 16	Preparation Example 7	24	0

[0059]

[0060] Examples 17 to 22. Preparation of Low-extrusion Compositions

[0061] Low-extrusion compositions were prepared by mixing ADM
5 according to Example 13 and purified water at the weight ratios shown in Table 3 below (hereinafter referred to as 'ADM composition or ADM').

[0062] [Table 3]

Classification	Purified water content (parts by weight) per 1 part by weight of ADM
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Example 17	50
Example 18	20
Example 19	10
Example 20	3
Example 21	2
Example 22	1

[0063]

[0064] Comparative Example 1: DMSO Treatment Group

[0065] Comparative Example 2: ADM 0% (only media)

[0066]

5 [0067] **Experimental Example 1: Maltitol Quantitative Analysis**

[0068] The contents of maltitol in the ADM compositions according to Examples 1 to 16 were measured and expressed as the maltitol contents (%) in Table 4. Among these, the sample photographs and detected maltitol contents of the ADM
10 compositions according to Examples 1 to 4 are shown in FIG. 3.

[0069] Referring to FIG. 3 and Table 4, it could be confirmed that maltitol was detected in the ADM compositions prepared according to the present disclosure under both 12-hour and
15 24-hour maltitol infiltration conditions.

[0070] Referring to FIGS. 3 and 4, it could be confirmed that

as the concentration of maltitol in the ADM composition increased, the content of maltitol remaining in the tissue powder increased, affecting the extrusion force, and since it was confirmed that the extrusion force decreased from Example 1 to Example 4, it could be seen that the average and maximum extrusion forces decreased depending on the maltitol content.

[0071]

[0072] **Experimental Example 2: Evaluation of Extrusion Force Depending on Whether There was Maltitol or not and Content of Maltitol**

[0073] To confirm the extrusion force characteristics depending on whether there was maltitol or not and content of maltitol, the ADM compositions according to Examples 1 to 16 were placed in a 3 cc syringe (Needle: 18G), the reagent was extruded from the syringe using a universal testing machine (UTM) at a speed of 50 mm/min and a constant force, and the results are shown in Table 4 and FIGS. 4 to 6. At this time, the average value was represented as an average extrusion force value observed during the total time interval of continuous extrusion of the reagent, and the maximum value was represented as a maximum extrusion force value observed during the total time interval of continuous extrusion of the reagent.

[0074] [Table 4]

Classif ication	Extrusion force average/max imum value (N)	Maltitol Content (%)	Classif ication	Extrusion force average/maxim um value (N)	Maltitol Content (%)
Example 1	55.2/157.3	Non- detection	Example 9	2.01/3.85	17.16
Example 2	4.57/11.44	6.23	Example 10	1.85/3.34	19.10
Example 3	2.86/6.30	9.79	Example 11	50.2/139.5	Non- detection
Example 4	2.85/6.23	10.68	Example 12	3.84/12.50	4.74
Example 5	2.83/4.18	Non- detection	Example 13	2.02/7.94	7.49
Example 6	2.20/3.90	4.74	Example 14	1.80/4.95	13.74
Example 7	1.44/3.66	7.49	Example 15	1.46/3.48	17.16
Example 8	1.94/3.90	13.74	Example 16	1.16/3.31	19.10

[0075] Referring to Table 4 and FIGS. 4 to 6, it could be

confirmed that as the concentration of maltitol in the ADM composition increased, the content of maltitol remaining in the tissue powder increased, which affected the extrusion force to decrease.

5 **[0076]** In addition, it could be confirmed that the low-extrusion compositions according to Examples of the present disclosure showed extrusion forces of 20 N or less in both a case where the maltitol solution was infiltration-treated for 12 hours (overnight) and a case where the maltitol
10 solution was infiltration-treated for 24 hours.

[0077] In addition, it could be confirmed that no significant difference occurred depending on whether maltitol was contained or not before sterilization referring to Examples 5 to 10 illustrated in FIG. 5, but a significant difference
15 occurred depending on whether maltitol was contained or not after sterilization for commercialization as shown in Examples 11 to 16 illustrated in FIG. 6. Further, it could be confirmed that the extrusion force increased rapidly during the sterilization process referring to Examples 5 and
20 11, which correspond to cases where maltitol was not contained, but the extrusion force remained at 20 N or less even after performing sterilization referring to Examples 6 to 10 and Examples 12 to 16, which correspond to cases where maltitol was contained.

[0078] That is, for the ADM compositions of Examples 1 and 11, which were sterilized and did not contain maltitol, both the average and maximum values were confirmed to have an extrusion force exceeding 20 N, but for the ADM compositions of Examples 12 to 16, which were sterilized and contained maltitol, both the average and maximum values were confirmed to have an extrusion force of less than 20 N. In other words, it could be confirmed that the extrusion force was shown to be significantly low in the concentration range of the maltitol solution of the present disclosure, which was greater than 0% and less than or equal to 25%.

[0079] In addition, the average extrusion force of the ADM composition generally increases during the sterilization process, but the results that the average extrusion force was rather decreased in Examples 14 to 16 could be confirmed.

[0080]

[0081] **Experimental Example 3: Evaluation of Wound Healing**

[0082] To confirm the wound healing effect depending on the contents of ADM prepared according to Examples of the present disclosure, the migration ability of human dermal fibroblasts (HDF) was evaluated through a scratch assay using Examples 17 to 22.

[0083] After creating scratches, cell migrations to the

scratch sites according to the passage of a period of 20 hours were observed, and the results are shown in FIGS. 7 and 8.

[0084] Referring to FIGS. 7 and 8, it was confirmed that cells
5 died and did not grow in the DMSO-treated group, but a wound healing effect was present in the ADM-treated group. That is, it was confirmed that a wound healing effect was present in all ranges of the ratio of ADM prepared according to Examples of the present disclosure to purified water, ranging
10 from 1:1-50. Among these, excellent effects were confirmed in the range of 1:2-50, and very excellent effects were confirmed in the range of 1:3-1:20.

[0085]

[0086] While exemplary embodiments of the present disclosure
15 have been described in detail above, the scope of rights of the present disclosure is not limited thereto, and various modifications and improvements made by those skilled in the art utilizing the basic concepts of the present disclosure defined in the following claims also fall within the scope
20 of rights of the present disclosure.

[0087] Unless otherwise defined, all technical terms used in the present disclosure have been used as the same meaning as commonly understood by those skilled in the art in the relevant fields of the present disclosure. The contents of

all publications incorporated in the present specification by reference are incorporated in the present disclosure by reference.

CLAIMS

【Claim 1】

A low-extrusion force composition comprising an acellular dermal matrix, comprising:

- 5 100 parts by weight of an acellular dermal matrix;
 100-3,000 parts by weight of water; and
 1-1,000 parts by weight of maltitol.

【Claim 2】

 The low-extrusion force composition of claim 1,
10 comprising an acellular dermal matrix having an extrusion
force of 20 N or less.

【Claim 3】

 A method for preparing a low-extrusion force composition
comprising an acellular dermal matrix, comprising the steps
15 of:

- (a) preparing a human-derived acellular dermal matrix
that has been deepidermized, defatted, and decellularized;
 (b) adding a maltitol solution to the acellular dermal
matrix to infiltrate it;
20 (c) freeze-drying the infiltration-completed acellular
dermal matrix and then pulverizing it; and
 (d) adding water to the pulverized acellular dermal
matrix to hydrate and paste it.

【Claim 4】

25 The method of claim 3, wherein in the step (b), the

maltitol solution is added in an amount of 20-40 parts by weight to 100 parts by weight of the acellular dermal matrix, and infiltration is performed for 10-30 hours.

【Claim 5】

5 The method of claim 3, wherein in the step (b), the maltitol solution is contained at a concentration of 20-40% in a mixed solution in which glycerol, propylene glycol, and a buffer solution are mixed.

【Claim 6】

10 The method of claim 3, wherein in the step (c), the pulverization is performed so that the proportion in which the ratio of the short axis to the long axis is 1:1-2,000 is 70% or more.

【Claim 7】

15 The method of claim 3, wherein in the step (d), water is added in an amount of 100-2,500 parts by weight per 100 parts by weight of the pulverized acellular dermal matrix.

【Claim 8】

20 The method of claim 3, further comprising a step (e) of performing sterilization by irradiating the paste-formed acellular dermal matrix with radiation at a dose in the range of 11-30 kGy after the step (d).