

METHOD FOR PRODUCING COLLAGEN MEMBRANE

Cross reference to related application

The present application claims the benefit of Chinese patent application No. 2023104239699 filed on April 19, 2023, which is incorporated herein by reference in its entirety as a part of this application.

Technical Field

The present invention relates to the technical field of processing a collagen, in particular to a method for producing a collagen membrane, especially relates to a collagen membrane suitable for periodontal regeneration and dural membrane repair and the preparation method thereof.

Background of the Invention

Collagen is a kind of natural fibrin that mainly exists in the skin, tendon, and bone of animals. It is also the most abundant functional protein in mammals, accounting for 25% to 30% of the total protein. Collagen is now widely used in the food industry, cosmetics, medical materials and other fields.

The connective tissue in the body is mainly composed of collagen, so collagen is often used as a membrane to replace the skin, blood vessels, ligaments and other connective tissues, and is widely used in the field of medical materials.

Current preparation method of collagen membrane is usually using oven drying method, and the method is: pouring the collagen liquid into the sterilized stainless steel tray, and putting the tray in the oven for drying. The collagen membrane dried by the method, however, often has situations such as uneven thickness, surface, insufficient tensile strength, which affect the quality of the product.

Therefore, improving the problems of uneven collagen membrane thickness and uneven surface, and enhancing the tensile strength can make the collagen membrane more applicable.

Summary of the Invention

In view of the above problems in the prior art, one of the objectives of the present invention is to provide a method for producing a collagen membrane, which can improve the problems of uneven collagen membrane thickness and uneven surface of the collagen membrane, and can

enhance the tensile strength of the collagen membrane.

After the research by the inventors of the present application, it was found that the vertical and horizontal tensile strength of the collagen membrane will be enhanced by using a dispensing machine for a double-layer bidirectional coating; and the problems of the uneven thickness and uneven surface will be improved by using a layer plate and pressed frame containing water-absorbing material, thus the defects in the traditional preparation method of the collagen membrane will be solved. The collagen membrane prepared by the present invention maintains the integrity of the collagen triple helix, and has high tensile strength, uniform thickness and smooth surface, which can be used to separate soft and hard tissues and accelerate tissue healing.

The present invention provides a method for producing collagen membrane, comprising the following steps:

S4: applying a defoamed collagen raw material to a layer plate by a dispensing machine; and

S5: placing a pressed frame on the defoamed collagen material after step S4.

In some embodiments, the defoamed collagen raw material is obtained by diluting and defoaming a collagen raw material.

In some embodiments, the collagen raw material is at least one selected from the group consisting of collagen extracted from animal tissue, and recombinant collagen.

In some embodiments, the form of the collagen raw material is solid, liquid, or a combination of solid and liquid.

In some embodiments, the method further comprises S6: performing heat pump drying on the defoamed collagen raw material to obtain a dried collagen membrane.

In some embodiments, the method further comprises S7: performing thermal crosslinking of the dried collagen membrane to obtain a semi-finished collagen membrane.

In some embodiments, the method further comprises S8: soaking the semi-finished collagen membrane in water to obtain a flat collagen membrane.

In some embodiments, the method further comprises S9: freeze-drying the flat collagen membrane to obtain the collagen membrane.

In some embodiments, the collagen raw material is preferably at least one selected from the group consisting of collagen extracted from animal tissue, and recombinant collagen.

In some embodiments, the form of the collagen raw material is solid, liquid, or a combination of solid and liquid.

In some embodiments, the solvent used for diluting the collagen raw material is preferably at least one selected from the group consisting of an organic solvent and an inorganic solvent.

Also, in terms of the organic solvent, an alcohol solvent such as a C1-C5 alcohol solvent is more ideal. Among the alcohol solvent, isopropanol is more ideal. For the inorganic solvent, water is more ideal.

In some embodiments, the concentration of the diluted collagen raw material is preferably 10 mg/g to 50 mg/g.

In some embodiments, in the above coating step, it is more ideal to use the dispensing machine for a double-layer bidirectional coating. Moreover, the inventors of this application speculate that the reasons why it is more ideal are as follows.

If the collagen raw material is only poured into the layer plate and dried, the fiber direction of the collagen will be arranged in an irregular direction, so the mechanical strength of the formed collagen membrane is poor. If the dispensing machine is used for double-layer bidirectional coating as in the present invention, the fibers of the collagen can be arranged in a specific direction, and the mechanical strength of the collagen membrane can be improved by the above method.

In some embodiments, the double-layer bidirectional coating means that the first layer is coated by using the dispensing machine in the left -right direction, and then the second layer is coated by using the dispensing machine in the up-down direction, that is, perpendicular to the coating direction of the first layer.

In some embodiments, the bottom of the layer plate preferably contains a water-absorbing material. Also, it is more preferable that the water-absorbing material is a porous inorganic material, or an organic polymer material. Also, the inventors of this application speculate that the reasons are as follows.

If there is no water-absorbing material at the bottom of the layer plate during the drying process of the collagen raw material, the water will flow in the drying process, so the thickness of the formed collagen membrane is not uniform.

If there is water-absorbing material at the bottom of the layer plate during the drying process of the collagen raw material, part of water can be moved to the water-absorbing material, which can effectively reduce the flow of water, so the thickness of the formed collagen membrane in this method is more uniform.

In some embodiments, it is ideal that the contact surface between the pressed frame and the

collagen raw material is covered with a net. Also, it is more ideal that the net is made of nylon material. The inventors of this application speculate that the reasons are as follows.

During the drying process of the collagen raw material, the pressed frame and the net will apply a weight to the collagen raw material, which can effectively reduce the excessive movement of collagen and water, and the nylon net is breathable, which will not affect the evaporation of water during the drying process, so the thickness of the formed collagen membrane is relatively uniform.

In some embodiments, the heat pump drying is preferably performed at a temperature of 40 °C to 75 °C.

In some embodiments, the heat pump drying is preferably performed for a drying time of 5 hours to 30 hours.

In some embodiments, the thermal crosslinking is preferably performed at a temperature of 60 °C to 90 °C.

In some embodiments, the thermal crosslinking is preferably performed for a time of 8 hours to 20 hours.

Description of the Drawings

FIG. 1 is a flowchart showing the method of the embodiment of the present invention.

FIG. 2 shows the appearance of the collagen membrane prepared in Example 1 of the present invention.

FIG. 3 shows the appearance of the collagen membrane prepared in Comparative Example 2 of the present invention.

Detailed Description of the Embodiments

Embodiments of the present invention will be described below.

Referring to FIG. 1, it is a flowchart showing the method for producing collagen membrane according to an embodiment of the present invention. As shown in FIG. 1, the method for producing the collagen membrane of the present invention comprises the following steps: providing a collagen raw material (S1); diluting the collagen (S2); defoaming the collagen raw material (S3); applying the collagen raw material to a layer plate by a dispensing machine (S4); placing a pressed frame on the collagen raw material (S5); performing heat pump drying on the

collagen raw material (S6); performing thermal crosslinking of the collagen membrane (S7); soaking the collagen membrane (S8); and drying the collagen membrane (S9).

In step (S1), the supplied collagen raw material can be collagen extracted from animal tissue, or recombinant collagen. For example, the collagen extracted from cowhide and beef tendon, or recombinant collagen produced by using yeast as genetically engineered carriers.

In one embodiment, the form of the collagen raw material is liquid, or solid, or a combination of solid and liquid. For example, it can be a collagen solution (liquid) dissolved in a solvent, a freeze-dried spongy-like collagen (solid), or a fibrous collagen slurry (solid-liquid mixture).

In step (S2), the solvent used for diluting the above collagen raw material contains but not limited to an organic solvent, an inorganic solvent and the combination thereof, such as isopropanol, water, etc.

Using water is ideal because the collagen raw material can be uniformly dispersed. If isopropanol is used, the collagen raw material can also be dispersed evenly, and isopropanol has antibacterial function, which is more ideal.

The concentration of the diluted collagen raw material, that is, the collagen raw material is preferably 10 to 50 mg/g per 1g of solvent. It is more preferably 15 to 45 mg/g, more preferably 20 to 40 mg/g, and particularly preferably 25 to 30 mg/g. Due to the viscosity of collagen in the solvent, if the concentration is more than 50mg/g, the viscosity is too high, which will make the operability poor. If the concentration is below 10mg/g, the mechanical strength of the membrane will be insufficient due to the low concentration of the collagen.

In step (S3), known methods may be used for defoaming, including but not limited to centrifugation, vacuuming, or a combination thereof. Among them, it is more ideal to use a centrifugal vacuum deoaming machine for defoaming, and the obtained material is uniform and free of bubbles.

In step (S4), if the known method that pouring the raw material into the layer plate for coating is used, the multilayer structure cannot be achieved, and the tensile strength of the collagen membrane is poor. On the contrary, if the dispensing machine is used for coating, multi-level and multi-direction coating can be achieved, and the tensile strength of the collagen membrane is higher.

Also, the dispensing machine of Pinxu Automation Equipment Co., LTD is used in the invention. The collagen raw material is poured into the material cylinder, the glue gun is located at

the origin, the coating path is inputted into the controller, and then the operation procedure is started.

Among them, if a double-layer bidirectional coating is applied, the tensile strength can be more ideal because the fiber of collagen is arranged vertically, and has a bilayer structure.

In step (S4), the coating is carried out by using a layer plate containing water-absorbing material, wherein the water-absorbing material is preferably a diatomite plate. The diatomite plate has a large number of small holes, which can achieve fast water absorption.

In step (S5), a pressed frame is placed above the collagen raw material after coating, and it is ideal that the pressed frame is covered with a nylon net. The nylon net can fix the shape of collagen raw material when drying, reduce the deformation after drying, and improve the problems of uneven membrane thickness and uneven surface.

Also, the pressed frame of the present invention is made of stainless steel, which has the same size as the layer plate, and is placed above after the collagen raw material is coated.

In step (S6), it is ideal that the heat pump drying is performed at a temperature of 40 to 75 °C. It is more preferably 45 °C to 70 °C, more preferably 50°C to 65 °C, and particularly preferably 55 °C to 60 °C. If the temperature is too low, the drying efficiency will be poor; on the other hand, if the temperature is too high, there will be a possibility of collagen denaturation. It is relatively ideal that the drying is performed for a drying time of 5 hours to 30 hours. It is more preferably 7 hours to 27 hours, more preferably 10 hours to 25 hours more ideal, and particularly preferably 15 to 20 °C. If the time is too short, the collagen raw material cannot be completely dried; on the other hand, if the time is too long, only the burden of time and cost will be increased.

In step (S7), it is ideal that the thermal crosslinking is performed at a temperature of 60°C to 90 °C. It is more preferably 63 °C to 87 °C, more preferably 66 to 84 °C, and particularly preferably 73 °C to 77 °C. If the temperature is too low, the thermal crosslinking effect will not be obvious; on the other hand, if the temperature is too high, there will be a possibility of collagen denaturation. It is ideal that the thermal crosslinking is performed for a time of 8 hours to 20 hours. It is more preferably 8 hours to 20 hours, more preferably 10 hours to 18 hours, and particularly preferably 13 hours to 15 hours. If the crosslinking time is too short, it will not be able to sufficiently crosslink, and the excellent effect of the invention cannot be obtained, if the crosslinking time is too long, only the burden of time and cost will be increased.

Further, the thermal crosslinking of the present invention refers to placing the collagen raw material into the heat pump drying oven and adjusting the heat pump drying oven to the target temperature and target time, and then stewing.

In step (S8), water for injection is used as a solvent of the water covering step. The so-called water covering step of step (S8) in the invention refers to soaking the collagen membrane in water. This step can reduce the ruffling of surface of the collagen membrane and increase the flatness of the collagen membrane.

In step (S9), the drying method can be heat pump drying, vacuum drying or freeze drying. Among them, during drying, freeze drying can retain the shape of the collagen membrane while drying, and the process is carried out at relatively low temperature, so it is more ideal.

The invention is specifically described by examples below, but these examples are only examples and do not mean that the invention is only limited to the states of these examples.

[Example 1]

A preparation method for collagen membrane, the specific steps are as follows:

- 1) The fibrous collagen was evenly mixed with water at a concentration of 24 mg/g.
- 2) Defoaming was performed using a centrifugal defoaming machine at a rotational speed of 2000rpm and a vacuum of -90 kPa for 5 minutes.
- 3) The defoamed collagen raw material was applied to a layer plate by a dispensing machine. The bottom of the layer plate had diatom mud plate as the water-absorbing material. A total of 2 layers were applied, and the directions were perpendicular to each other.
- 4) A pressed frame was placed on the defoamed collagen raw material, and the contact surface between the pressed frame and the defoamed collagen raw material was covered with a nylon net.
- 5) The defoamed collagen raw material was subjected to heat pump drying, at a temperature of 45°C for 20 hours. After this step, the collagen raw material basically became a membrane.
- 6) The collagen dried membrane was subjected to thermal crosslinking as described above, at a temperature of 70°C for 16 hours to obtain a collagen semi-finished membrane.
- 7) The collagen semi-finished membrane was soaked in injection water and covered with water to flatten the surface of membrane.
- 8) The collagen membrane covered with water was subjected to freeze-drying by placing in a freeze-drying machine to obtain the collagen membrane.

[Example 2]

A preparation method for collagen membrane, the specific steps are as follows:

- 1) The fibrous collagen was evenly mixed with water at a concentration of 35 mg/g.
- 2) Defoaming was performed using a centrifugal defoaming machine at a rotational speed of 2000rpm and a vacuum of -90 kPa for 5 minutes.
- 3) The defoamed collagen raw material was applied to a layer plate by a dispensing machine. The bottom of the layer plate had plasterboard as the water-absorbing material. A total of 2 layers were applied, and the directions were perpendicular to each other.
- 4) A pressed frame was placed on the defoamed collagen raw material, and the contact surface between the pressed frame and the defoamed collagen raw material was covered with a nylon net.
- 5) The defoamed collagen raw material was subjected to heat pump drying, at a temperature of 60°C for 12 hours. After this step, the collagen raw material basically became a membrane.
- 6) The collagen drying membrane was subjected to thermal crosslinking as described above, at a temperature of 80°C for 12 hours to obtain a collagen semi-finished membrane.
- 7) The collagen semi-finished membrane was soaked in injection water and covered with water to flatten the surface of membrane.
- 8) The collagen membrane covered with water was subjected to freeze-drying by placing in a freeze-drying machine to obtain the collagen membrane.

[Comparative Example 1]

The collagen membrane was prepared in the same way as in Example 1, except that the collagen raw material was poured directly into the layer plate instead of using a dispensing machine for coating.

[Comparative Example 2]

The collagen membrane was prepared in the same way as in Example 1, except that the water-absorbing material was not used as chassis and the pressed frame was not placed above the collagen raw material.

[Comparative Example 3]

The collagen membrane was prepared in the same way as in Example 1, except that the heat pump drying is performed at a temperature of 80°C.

[Comparative Example 4]

The collagen membrane was prepared in the same method as in Example 1, except that the coating step was performed using the dispensing machine for a single-layer unidirectional coating.

The average thickness, the thickness standard deviation, the triple helix structure, and the tensile strength of the collagen membrane obtained from Example 1, Example 2 and Comparing Examples 1 to 4 were evaluated as described below, and the results thereof were shown in Table 1 below.

<Average thickness>

The average thickness was determined by calculating the average value of the collagen membrane by measuring the thickness of 20 points by the thickness meter.

<Thickness standard deviation>

The of the thickness standard deviation was calculated from the thickness measurement of the collagen membrane at 20 points measured by the thickness meter.

<Triple helix structure>

The triple helix structure referred to using electrophoresis for protein separation, and using $\alpha 1$ and $\alpha 2$ bands to determine whether the triple helix structure was complete.

<Tensile strength>

Tensile strength was conducted by the microcomputer controlled electronic universal material testing machine of Haixiqiang Instrument Manufacturing Co., LTD., the test was conducted by cutting the collagen membrane into the size of 5 * 15mm, fixing the long side on the tensile fixture, at a moving rate of the fixture of 1mm/min. The tensile strength was obtained by dividing the maximum load weight from the cross-sectional area of the sample.

Table 1

	Example 1	Example 2	Comparative Example 1	Comparative Example 2	Comparative Example 3	Comparative Example 4
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Average thickness	0.278 mm	0.285 mm	0.288 mm	0.275 mm	0.301 mm	0.283 mm
Thickness standard deviation	0.019 mm	0.018 mm	0.021 mm	0.046 mm	0.024 mm	0.020 mm
Triple helix structure	complete	complete	complete	complete	not complete	complete
Tensile strength	36.01 MPa	34.42 MPa	22.68 MPa	27.35 MPa	13.20 MPa	29.45 MPa

Also, in order to better understand the flatness difference of the membranes in Example 1 and Comparative Example 2, their appearance results were also displayed in FIGs. 2 and 3.

<Comparison with the commercial product described>

For Example 1 and the commercial products, the tensile strength was measured separately in the manner as described above, and the results were shown in Table 2.

Among them, the trade name of commercial product 1 was Remaix made by Matricel company, the trade name of commercial product 2 was Bio-Gide made by Geistlich Biomaterials company, and the trade name of commercial product 3 was Ossix Plus made by Datum Dental Biotech company.

Table 2

	Example 1	Commercial product 1 Remaix	Commercial product 2 Bio-Gide	Commercial product 3 Ossix Plus
Tensile strength (MPa)	36.01	10.4	4.6	5.13

From the results of Table 1, it can be found that the collagen membrane prepared by satisfying the process conditions of the present invention had a lower standard deviation of thickness compared with the Comparative Example 2 which did not satisfy the process conditions of the invention, that is, it had a better film flatness.

From the results of Table 1, it can also be found that the collagen membrane prepared by satisfying the process conditions of the present invention had a complete collagen triple helix structure compared with the Comparative Example 3 which did not satisfy the process conditions of the present invention. From the results of Table 1 and Table 2, it can be found that the collagen membrane prepared by satisfying the process conditions of the present invention had more sufficient physical strength whether compared with the Comparative Example 1 which did not satisfy the process conditions of the present invention or compared with the commercial products.

In conclusion, the collagen membrane prepared according to the present invention has the following advantages:

(1) Collagen is a heat-sensitive substance. If the temperature in the drying process is too high, it will lead to the destruction of the triple helix structure and its original effect will be lost. The drying temperature in the present invention is controlled within a specific range, which can avoid the destruction of the triple helix structure in the collagen, and maintain the collagen integrity.

(2) The collagen membrane prepared according to the present invention, due to the action of the pressed frame and the water-absorbing material, the thickness of the dried collagen membrane is relatively uniform, and the uneven surface and wrinkles are reduced.

(3) The collagen membrane prepared according to the present invention effectively increases the tensile strength of the collagen membrane due to the action of the dispensing machine.

[Symbol Description]

S1 to S9: Step.

What is claimed is:

1. A method for producing collagen membrane, comprising the following steps:

S4: applying a defoamed collagen raw material to a layer plate by a dispensing machine; and

S5: placing a pressed frame on the defoamed collagen raw material after step S4.

2. The method according to claim 1, wherein the defoamed collagen raw material is obtained by diluting and defoaming a collagen raw material;

optionally, the collagen raw material is at least one selected from the group consisting of collagen extracted from animal tissue, and recombinant collagen;

optionally, the form of the collagen raw material is solid, liquid, or a combination of solid and liquid.

3. The method according to claim 1 or 2, wherein the method further comprises S6: performing heat pump drying on the defoamed collagen raw material to obtain a dried collagen membrane;

optionally, the method further comprises S7: performing thermal crosslinking of the collagen dried membrane to obtain a semi-finished collagen membrane;

optionally, the method further comprises S8: soaking the semi-finished collagen membrane in water to obtain a flat collagen membrane; and

optionally, the method further comprises S9: freeze-drying the flat collagen membrane to obtain the collagen membrane.

4. The method according to claim 2, wherein the solvent used for the diluting step is at least one selected from the group consisting of an organic solvent and an inorganic solvent; and/or the concentration of the diluted collagen raw material is 10 mg/g to 50 mg/g.

5. The method according to claim 4, wherein the organic solvent is a C1-C5 alcohol solvent; and/or the inorganic solvent is water.

6. The method according to claim 4, wherein the organic solvent is isopropanol.

7. The method according to claim 1 or 2, wherein the coating step is performed by using the dispensing machine for a double-layer bidirectional coating; and/or

the bottom of the layer plate contains a water-absorbing material; and/or

the contact surface between the pressed frame and the defoamed collagen raw material is covered with a net.

8. The method according to claim 7, wherein the water-absorbing material is at least one selected from the group consisting of a porous inorganic material, and an organic polymer material.

9. The preparation method according to claim 7, wherein the net is made of nylon material.

10. The method according to any one of claims 3 to 9, wherein the heat pump drying is performed at a temperature of 40 °C to 75 °C, and/or the heat pump drying is performed for a drying time of 5 hours to 30 hours; and/or

the thermal crosslinking is performed at a temperature of 60 °C to 90 °C, and/or the thermal crosslinking is performed for a time of 8 hours to 20 hours.