

PREPARATION METHOD FOR BIOLOGICAL SURGERY PATCH, BIOLOGICAL SURGERY PATCH PREPARED BY METHOD, AND APPLICATION THEREOF

Cross-reference to related application

The present application claims priority to Chinese Patent Application No. 201910584735.6, entitled “Preparation method for biological surgery patch, biological surgery patch prepared by method, and application thereof”, and filed on July 1, 2019, the entire disclosure of which is incorporated herein by reference in its entirety.

Technical Field

The present invention relates to the technical field of biological materials, in particular to a preparation method for a biological surgery patch, a biological surgery patch prepared based on the method and application thereof.

Background Art

The existing method for preparing the biological surgery patch is direct fermentation, and is a static fermentation method without adding other ingredients during the fermentation process, the thickness of the obtained bacterial cellulose membrane is controlled according to the length of the fermentation time, and thereby indirectly adjusting the mechanical properties of the membrane material. Solid state fermentation takes a long time, the improvement of the mechanical properties of the membrane material is not obvious, and the pore size is dense, which is not conducive to the proliferation of cells and tissues, and it is difficult to meet clinical needs.

Summary of the Invention

One purpose of the present invention is to provide a preparation method of a biological surgery patch, a biological surgery patch prepared based on the method and application thereof. The biological surgery patch obtained by the preparation method described by the present invention is good in biocompatibility, mechanical properties, sewing performance and clinical application convenience, and capable of satisfying the basic requirements of surgery patch materials.

The present invention provides a preparation method for a biological surgery patch, comprising the following steps:

(1) fermenting culturing a strain to obtain a bacterial cellulose membrane; adding biopolymer materials during the fermenting culturing process; the biopolymer materials include

one or more of polylactic acid, polyamide, polyester, glycerol, polylactide, polycaprolactone, polyvinyl chloride and polyethylene;

(2) soaking the bacterial cellulose membrane obtained in step (1) in an inorganic base solution and an acid solution sequentially to obtain a purified bacterial cellulose membrane;

(3) immersing the purified bacterial cellulose membrane obtained in step (2) into an oxidant solution for oxidation to obtain an oxidized bacterial cellulose membrane;

(4) subjecting the oxidized bacterial cellulose membrane obtained in step (3) to freeze-thawing to obtain a freeze-thawed bacterial cellulose membrane;

(5) placing the freeze-thawed bacterial cellulose membrane obtained in step (4) into a glycerol aqueous solution, stirring and drying to obtain a composite processed bacterial cellulose membrane;

(6) performing electrostatic spinning on the composite processed bacterial cellulose membrane obtained in step (5) to obtain an intermediate product of a biological surgery patch; the electrostatic spinning solution includes a solution of one or more of polylactic acid, polycaprolactone, chitosan, polyacrylonitrile and polycarbonate; the solvent of the solution is one or more of dichloromethane, trichloromethane, tetrahydrofuran, N,N-dimethylformamide, N,N-dimethylacetamide, dimethyl sulfoxide, diethyl ether, n-hexane and petroleum ether; and

(7) subjecting the intermediate product of a biological surgery patch obtained in step (6) to freeze drying and membrane pressing to obtain the biological surgery patch.

Preferably, each 1000 mL of the medium for the fermenting culturing in step (1) comprises: 20 g to 25 g of mannitol, 10 g to 15 g of polyethylene glycol, 25 g to 30 g of sucrose, 15 g to 20 g of glucose, 0.5 mg to 0.8 mg of nicotinic acid, 5 g to 6 g of yeast powder, 10 g to 15 g of dry powder of corn steep liquor, 5 g to 10 g of beef extract, 5 g to 10 g of peptone, 1.5 g to 2.5 g of KH_2PO_4 , 2.0 g to 2.5 g of MgSO_4 , 8 g to 10 g of anhydrous ethanol, 0.5 g to 0.8 g of ammonium sulfate, 0.5 g to 0.6 g of citric acid, 0.5 g to 0.6 g of sodium carboxymethylcellulose and 40 g to 50 g of a mixture of gluconic acid and sodium gluconate; and the mass ratio of the gluconic acid to the sodium gluconate is 1: (1 to 5).

Preferably, the strain of step (1) comprises one or more of *Acetobacter*, *Rhizobium*, *Gluconobacter*, *Acetobacter xylinum*, *Sarcina*, *Pseudomonas*, *Achromobacter*, *Alcaligenes*, *Aerobacter*, *Azotobacter*, and *Agrobacterium*.

Preferably, the fermenting culturing in step (1) is performed at 20°C to 40°C with an

oxygen flux of 0.01 L/min to 10 L/min and a pH value of 3 to 9 for 6 days to 14 days.

Preferably, when the biopolymer materials in step (1) include two or more types, the biopolymer materials are added one by one in the order of polylactic acid, polyamide, polyester, glycerol, polylactide, polycaprolactone, polyvinyl chloride, and polyethylene.

Preferably, the oxidant in step (3) is an aqueous solution comprising one or more hydrogen peroxide, sodium hypochlorite, sodium peroxide, potassium peroxide, peracetic acid, sodium percarbonate, sodium perborate and potassium perborate.

Preferably, the mass fractions of the hydrogen peroxide, sodium hypochlorite, sodium peroxide, potassium peroxide, peroxyacetic acid, sodium percarbonate, sodium perborate and potassium perborate in water are 1% to 50%, respectively.

Preferably, in step (4), the freeze-thawing is carried out as follows: freezing the oxidized bacterial cellulose membrane obtained in step (3) at -45°C to -1°C for 0.2 to 10 hours, taking out and thawing under the environment of 5°C to 35°C for 0.2 to 10 hours, and discarding the thawed water; repeating the above freezing and thawing operations for 2 to 9 times.

Preferably, the mass concentrations of polylactic acid, polycaprolactone, chitosan, polyacrylonitrile or polycarbonate in the electrostatic spinning solution in step (6) are 0.01 g/mL to 5 g/mL, respectively.

Preferably, the mass ratios of the composite processed bacterial cellulose membrane in step (6) to polylactic acid, polyacrylonitrile, polycarbonate, polyvinylidene fluoride, chitosan or polycaprolactone are (0.1 to 3):10, respectively.

The present invention also provides a biological surgery patch prepared by the preparation method according to the above technical solutions.

The biological surgery patch described by the present invention is composed of bacterial cellulose and has a nano-network structure.

Preferably, the dry strength and wet strength of the bacterial cellulose are high, and the Young's modulus of its microfibers may be 10 GPa to 1000 GPa (preferably 50 GPa to 200 GPa), and the tensile strength may be 0.1 GPa to 100 GPa (preferably 1 GPa to 20 GPa, more preferably 2 GPa to 10 GPa).

Wherein, the unit of the bacterial cellulose is protofibril which has a diameter of 0.1 nm to 50 nm (preferably 1.0 nm to 10 nm), the protofibril is formed by binding a plurality of β -1,4-glucans, and has a triple helix structure. These protofibrils are aggregated to form

microfibrils, and the microfibrils are further aggregated into assembled bundle fibers with a ribbon structure, thereby forming the nano-network structure of the present invention.

In one embodiment of the present invention, the microfibrils have a diameter of 0.1 nm to 20 nm. In another embodiment of the present invention, the fibers in the nano-network structure (i.e., the assembled bundle fibers) have a diameter of 10 nm to 200 nm. The assembled bundle fibers are equivalent in size to the bundle of collagen fibers.

Wherein, the assembly process of cellulose chains (i.e., the above assembled bundle fibers) is a significant dynamic process, and the porosity and pore size of the obtained nano-network structure can be adjusted and controlled within a certain range. In one embodiment of the present invention, the porosity of the nano-network structure is 60% to 99%; and the pore size of the nano-network structure is 10 nm to 500 nm.

The biological surgery patch is prepared by using a modern biological fermentation technology and has excellent mechanical properties and biological properties. In addition, the biological surgery patch is good in biocompatibility, is a pure cellulose material, and non-animal-derived, has no immunogenicity risk, no organic solvent residual risk, is excellent in biocompatibility, and capable of satisfying various clinical requirements.

In another aspect of the present invention, the use of the biological surgery patch described by the present invention for the preparation of repair material for medical use is also provided. The medical use of the present invention includes, but are not limited to, neurosurgery (dura mater), general surgery (abdominal wall hernia, abdominal wall defect, inguinal hernia, breast patch, pelvic floor patch and the like), and cardiovascular department (atrial septum, ventricular septum). Specifically, the biological surgery patch described by the present invention can be used as a dura mater, a breast patch, a pelvic floor patch, an atrial septum, a ventricular septum, and the like, or can be used as a repair material for treating abdominal wall hernia, abdominal wall defect, and inguinal hernia.

The present invention provides a method for preparing a biological surgery patch. The base material of the preparation method is as follows: preparing a bacterial cellulose membrane by biological fermentation; subjecting the bacterial cellulose membrane prepared by biological fermentation to purification, oxidation, freeze thawing, composite process processing, electrostatic spinning, freeze drying and membrane pressing to obtain the biological surgery patch. In the present invention, the bacterial cellulose membrane prepared by fermentation of

microorganisms through a static/dynamic method has the properties such as tensile strength and the stitching strength that meet the clinical requirements on biological surgery patches by adding a proper amount of biopolymer materials during the fermenting culturing process. The bacterial cellulose membrane has improved pore size of the material by a chemical method and can promote the proliferation of cell tissues. After the bacterial cellulose membrane is covered with a biopolymer coating layer with antibacterial and degradable functions on the surface through an electrostatic spinning process, the obtained biological surgery patch can play a role of bacteria inhibition, infection prevention and tissue adhesion prevention, which is of great significance for the rapid recovery of patients after operation. Compared with other animal-derived base materials, the biological surgery patch described by the present invention uses bacterial cellulose membrane as a base material, and has the advantages of wide sources, low cost and safer clinical application. According to the present invention, the thickness and the mechanical properties of the obtained bacterial cellulose membrane can better meet with the requirements for clinical use through the improvement of fermentation conditions, scaffold materials that are more suitable for the growth of human tissue cells can be obtained by the chemical treatment and freeze-thaw drying methods, and the biological patch obtains the effects of anti-bacteria and adhesion prevention by electrostatic spinning, so that the effects of greatly improving stitching strength and biocompatibility are produced. The biological surgery patch obtained by the preparation method of the present invention is good in biocompatibility, mechanical properties, sewing performance and clinical application convenience, and capable of satisfying the basic requirements of surgery patch materials.

Brief Description of the Drawings

Figure 1 is a physical image of a biological surgery patch provided by the present invention (after being cut);

Figure 2 is a SEM image (5000-fold magnification) of a biological surgery patch provided by the present invention.

Specific Modes for Carrying Out the Embodiments

The present invention provides a method for preparing a biological surgery patch, the base material for preparation is a bacterial cellulose membrane prepared by biological fermentation; and the bacterial cellulose membrane prepared by biological fermentation is subjected to purification, oxidation, freeze thawing, composite process processing, electrostatic spinning,

freeze drying and membrane pressing to obtain the biological surgery patch.

The preparation method of the biological surgery patch comprises the following steps:

(1) fermenting culturing a strain to obtain a bacterial cellulose membrane; adding biopolymer materials during the fermenting culturing process; the biopolymer materials include one or more of polylactic acid, polyamide, polyester, glycerol, polylactide, polycaprolactone, polyvinyl chloride and polyethylene;

(2) soaking the bacterial cellulose membrane obtained in step (1) in an inorganic base solution and an acid solution sequentially to obtain a purified bacterial cellulose membrane;

(3) immersing the purified bacterial cellulose membrane obtained in step (2) in an oxidant solution for oxidation to obtain an oxidized bacterial cellulose membrane;

(4) subjecting the oxidized bacterial cellulose membrane obtained in step (3) to freeze-thawing to obtain a freeze-thawed bacterial cellulose membrane;

(5) placing the freeze-thawed bacterial cellulose membrane obtained in step (4) into a glycerol aqueous solution, stirring, and drying to obtain a composite processed bacterial cellulose membrane;

(6) performing electrostatic spinning on the composite processed bacterial cellulose membrane obtained in step (5) to obtain an intermediate product of a biological surgery patch; the electrostatic spinning solution includes a solution of one or more of polylactic acid, polycaprolactone, chitosan, polyacrylonitrile and polycarbonate; the solvent of the solution is one or more of dichloromethane, trichloromethane, tetrahydrofuran, N,N-dimethylformamide, N,N- dimethylacetamide, dimethyl sulfoxide, diethyl ether, n-hexane and petroleum ether; and

(7) subjecting the intermediate product of a biological surgery patch obtained in step (6) to freeze drying and membrane pressing to obtain the biological surgery patch.

In the present invention, the strain is subjected to fermentation culture to obtain a bacterial cellulose membrane; the biopolymer materials are added during the fermenting culturing process; the biopolymer materials include one or more of polylactic acid, polyamide, polyester, glycerol, polylactide, polycaprolactone, polyvinyl chloride and polyethylene. In the present invention, the strain is preferably subjected to activation and amplification culture to obtain a seed liquid, the seed liquid is inoculated into a fermentation medium for fermentation culture to obtain a bacterial cellulose membrane; the strain preferably comprises one or more of *Acetobacter*, *Rhizobium*, *Gluconobacter*, *Acetobacter xylinum*, *Sarcina*, *Pseudomonas*, *Achromobacter*,

Alcaligenes, *Aerobacter*, *Azotobacter*, and *Agrobacterium*; the fermenting culturing is preferably carried out under the conditions of 20°C to 40°C with an oxygen flux of 0.01 L/min to 10 L/min and a pH value of 3 to 9 for 6 days to 14 days, and mannitol a of 1% to 5% by volume and polyethylene glycol of 1% to 10% by volume of the fermentation liquid are respectively added on the first day and the third day of the fermentation. The biopolymer materials are added in the fermenting culturing process. The biopolymer materials include one or more of polylactic acid, polyamide, polyester, glycerol, polylactide, polycaprolactone, polyvinyl chloride and polyethylene. In the present invention, when the biopolymer materials include a plurality of types, they are preferably added one by one in the order of polylactic acid, polyamide, polyester, glycerol, polylactide, polycaprolactone, polyvinyl chloride, and polyethylene. For example, when the biopolymer materials include polyester and polyvinyl chloride, polyester is added first, and then polyvinyl chloride is added; and when the biopolymer materials include glycerol, polycaprolactone and polyethylene, glycerol is added first, then polycaprolactone is added, and finally polyethylene is added. In the present invention, the addition of the biopolymer materials can accelerate the fermentation of the bacterial cellulose membrane and enhance the mechanical properties and the microporous structure of the bacterial cellulose membrane at the same time, so that the bacterial cellulose membrane meets various clinical requirements. In the present invention, each 1000 mL of the medium for the fermenting culturing comprises 20 g to 25 g of mannitol, 10 g to 15 g of polyethylene glycol, 25 g to 30 g of sucrose, 15 g to 20 g of glucose, 0.5 mg to 0.8 mg of nicotinic acid, 5 g to 6 g of yeast powder, 10 g to 15 g of dry powder of corn steep liquor, 5 g to 10 g of beef extract, 5 g to 10 g of peptone, 1.5 g to 2.5 g of KH_2PO_4 , 2.0 g to 2.5 g of MgSO_4 , 8 g to 10 g of anhydrous ethanol, 0.5 g to 0.8 g of ammonium sulfate, 0.5 g to 0.6 g of citric acid, 0.5 g to 0.6 g of sodium carboxymethylcellulose and 40 g to 50 g of a mixture of gluconic acid and sodium gluconate; the mass ratio of the gluconic acid to the sodium gluconate is 1:(1 to 5), more preferably 1:3.5. In the present invention, the fermenting culturing is preferably preformed at 30°C with an oxygen flux of 1.0 L/min and a pH of 6.0 for 8 days. In the present invention, the bacterial cellulose membrane has a thickness of 0.05 cm to 2.0 cm, more preferably 0.5 cm.

In the present invention, after the bacterial cellulose membrane is obtained, the bacterial cellulose membrane is sequentially soaked in an inorganic base solution and an acid solution to obtain a purified bacterial cellulose membrane. In the present invention, the bacterial cellulose

membrane is preferably firstly subjected to a first cleaning, the bacterial cellulose membrane after the first cleaning is soaked in an inorganic base solution for 0.5 h to 5 h, heated and boiled for 1 h to 5 h, and then subjected to a second cleaning, soaked in an acid solution for 0.5 h to 5 h, and subjected to a third cleaning to obtain a purified bacterial cellulose membrane; the first cleaning, the second cleaning and the third cleaning are independently soaking and stirring in the water for injection for 3 to 60 min, and then the water for injection is discarded. In the present invention, the number of times of the first cleaning, the second cleaning, and the third cleaning is preferably 5 times. In the present invention, the first washing is preferably as follows: washing the bacterial cellulose membrane to a pH of 4.0 to 8.0, more preferably 6.0. In the present invention, the inorganic base solution comprises one or more of sodium hydroxide solution, potassium hydroxide solution, barium hydroxide solution, calcium hydroxide solution, aluminum hydroxide solution, lithium hydroxide solution, magnesium hydroxide solution, zinc hydroxide solution, sodium carbonate solution and sodium bicarbonate solution. The mass concentration of the inorganic base solution is 0.1 mol/L to 5.0 mol/L, and the temperature is 60°C to 100°C. In the present invention, the acid solution includes one or more of organic acids such as acetic acid, citric acid, lactic acid, oxalic acid, formic acid, palmitic acid, oleic acid, linoleic acid, and linolenic acid.

In the present invention, after the purified bacterial cellulose membrane is obtained, the purified bacterial cellulose membrane is immersed into an oxidant solution for oxidation to obtain an oxidized bacterial cellulose membrane. In the present invention, the purified bacterial cellulose membrane is preferably immersed into an oxidant solution, and stirred for 5 to 60 min, and then the resultant is subjected to vacuum filtration and leaching to obtain an oxidized bacterial cellulose membrane. In the present invention, the oxidant is one or more of aqueous solutions of hydrogen peroxide, sodium hypochlorite, sodium peroxide, potassium peroxide, peracetic acid, sodium percarbonate, sodium perborate and potassium perborate with the mass fractions of 1% to 50%, respectively. In the present invention, the oxidation enables the pore size of the bacterial cellulose membrane to meet the growth and reproduction requirements of tissue cells and promotes tissue growth. In the present invention, the vacuum filtration is preferably as follows: loading the bacterial cellulose membrane in a glass column, starting a vacuum pump for suction filtration, and preferably leaching the bacterial cellulose membrane with water for injection after suction filtration. In the present invention, the leaching is performed until the

content of bacterial endotoxin in the solution for leaching is 0.03EU/ml or less. In the present invention, the leaching is preferably as follows: washing the bacterial cellulose membrane to a pH of 4.0 to 8.0, more preferably 6.0.

In the present invention, after the oxidized bacterial cellulose membrane is obtained, the oxidized bacterial cellulose membrane is subjected to freeze-thawing to obtain a freeze-thawed bacterial cellulose membrane. In the present invention, the oxidized bacterial cellulose membrane is preferably frozen at a temperature of -45°C to -1°C for 0.2 to 10 hours, taken out and thawed under the environment of 5°C to 35°C for 0.2 to 10 hours, and the thawed water is discarded; the freezing and thawing operations are repeated for 2 to 9 times, the bacterial cellulose membrane which is thawed for the last time is taken out, and the thawed water is discarded to obtain a freeze-thawed bacterial cellulose membrane.

In the present invention, after the freeze-thawed bacterial cellulose membrane is obtained, the freeze-thawed bacterial cellulose membrane is placed into a glycerol aqueous solution, stirred and dried to obtain a composite processed bacterial cellulose membrane according to the present invention. Preferably, in the present invention, the freeze-thawed bacterial cellulose membrane is placed into a glycerol aqueous solution with a mass fraction of 0.5% to 3%, and stirred under 20 rpm to 1000 rpm for 0.5 h to 4 h. In the present invention, the bacterial cellulose membrane is preferably spread on a stainless steel mesh for drying, and the composite processed bacterial cellulose membrane is obtained after drying. In the present invention, the drying is performed at 10°C to 99°C for 0.5 h to 24 h, more preferably 65°C . In the present invention, the mass fraction of glycerol in the aqueous glycerol solution is preferably 3.85%. In the present invention, the stirring is preferably performed at 20 rpm to 1000 rpm for 0.5 h to 4 h, most preferably at 500 rpm for 4 h. In the present invention, the area of the stainless steel mesh is preferably 20 cm to 100 cm * 20 cm to 100 cm, and the mesh number is preferably 100 meshes to 50 meshes. When spread according to the present invention, it is preferable to ensure that the composite membrane material is smooth and no air bubbles are generated between the composite membrane material and the stainless steel mesh. In the present invention, the drying method is not particularly limited, and it is preferable to perform the drying in a blast drying oven. The bacterial cellulose membrane after the composite processing in the present invention is a biological surgery patch substrate, and the biological patch substrate in the present invention preferably has a fiber diameter of 0.05 μm to 0.5 μm ; preferably a thickness of 2 μm to 2000 μm , a size of “a” cm * “b”

cm ($2 \leq a \leq 10$, $2 \leq b \leq 10$), and preferably a shape of rectangular, circular, elliptical or irregular.

In the present invention, after the composite processed bacterial cellulose membrane is obtained, the composite processed bacterial cellulose membrane is electrostatically spun to obtain an intermediate product of a biological surgery patch; the electrostatic spinning solution includes a solution of one or more of polylactic acid, polycaprolactone, chitosan, polyacrylonitrile and polycarbonate; the solvent of the solution is one or more of dichloromethane, trichloromethane, tetrahydrofuran, N,N-dimethylformamide, N,N-dimethylacetamide, dimethyl sulfoxide, diethyl ether, n-hexane and petroleum ether. In the present invention, the composite processed bacterial cellulose membrane is preferably fixed on a receiving device of an electrostatic spinning device, the electrostatic spinning solution is filled into a syringe for electrostatic spinning, to obtain an intermediate product of a biological surgery patch. The electrostatic spinning solution comprises a solution comprising one or more of polylactic acid, polycaprolactone, chitosan, polyacrylonitrile and polycarbonate with a mass concentration of 0.01 g/mL to 5 g/mL. The solvent of the solution is one or more of dichloromethane, trichloromethane, tetrahydrofuran, N,N-dimethylformamide, N,N-dimethylacetamide, dimethyl sulfoxide, diethyl ether, n-hexane and petroleum ether. The mass ratios of the bacterial cellulose membrane after freeze-thawing and drying to polylactic acid, polyacrylonitrile, polycarbonate, polyvinylidene fluoride, chitosan or polycaprolactone are (0.1 to 3):10, respectively; the electrostatic spinning is performed at a positive electrode voltage of 10 to 30 KV, a negative electrode voltage of -0.1 to -10 KV, and a receiving distance of 5 cm to 30 cm. In the present invention, the electrostatic spinning solution preferably comprises a solution comprising one or more of polylactic acid, polycaprolactone, chitosan, polyacrylonitrile, and polycarbonate with a mass concentration of 0.2 g/mL to 5 g/mL. In the present invention, when the electrostatic spinning solution is a polylactic acid-dichloromethane solution and a polycaprolactone-dichloromethane solution, the polylactic acid-dichloromethane solution is preferably transferred into a syringe for electrostatic spinning to obtain a biological surgery patch intermediate product A1, the polycaprolactone-dichloromethane solution is then transferred into the syringe for electrostatic spinning to obtain a biological surgery patch intermediate A2; in the present invention, the use order of different electrostatic spinning solutions is not limited, for example, the electrostatic spinning order of the polylactic acid-dichloromethane solution and the polycaprolactone-dichloromethane solution may be changed. In the present invention, the mass

ratio of the bacterial cellulose membrane after freeze-thawing and drying to polylactic acid, polyacrylonitrile, polycarbonate, polyvinylidene fluoride, chitosan or polycaprolactone is preferably 1:10, 1:8, 1:8, 1:10, 1:5 and 1:8, respectively. In the present invention, the electrostatic spinning is preferably performed at a positive electrode voltage of 20 KV, a negative electrode voltage of 2 KV, and a receiving distance of 15 cm. After the bacterial cellulose membrane provided by the present invention is covered with a biopolymer coating layer with antibacterial and degradable functions on the surface through an electrostatic spinning process, the obtained biological surgery patch can play a role of bacteria inhibition, infection prevention and tissue adhesion prevention.

In the present invention, after the intermediate product of the biological surgery patch is obtained, the biological surgery patch intermediate product is subjected to freeze drying and membrane pressing to obtain a biological surgery patch. In the present invention, the intermediate product of the biological surgery patch is preferably soaked in an ethanol aqueous solution with a volume concentration of 50% to 99% for 10 to 60 min, then taken out and immersed into water for injection for soaking for 10 to 60 min, and freeze-dried to obtain a freeze-dried intermediate product of the biological surgery patch. After the freeze-dried biological surgery patch intermediate product is obtained, the biological surgery patch intermediate product after the freeze-drying is subjected to membrane pressing to obtain a biological surgery patch. In the present invention, the thickness of the pressed membrane is 0.01 mm to 10 mm. In the present invention, the biological surgery patch is preferably cut into a specification of 5 mm to 100 mm $\times$ 5 mm to 100 mm, and the biological surgery patch product is obtained after inner packing, outer packing, sterilization, and packaging. In the present invention, the sterilization method preferably includes one or more of microwave, ultraviolet, X-ray, γ -ray, electron beam, and Co60 sterilization methods, and when the sterilization method is Co60, the dose is preferably 5 to 50 KGy, more preferably 25 KGy.

The present invention also provides a biological surgery patch prepared base on the preparation method described in the above technical solutions. The physical image of the biological surgery patch is shown in Fig. 1, and the SEM image is shown in Fig. 2. In the present invention, the bacterial cellulose membrane prepared by fermentation of microorganisms through a static/dynamic method has the properties such as tensile strength, the stitching strength that meet the clinical requirements on biological surgery patches by adding a proper amount of

biopolymer materials during the fermenting culturing process. The bacterial cellulose membrane has improved pore size of the material by a chemical method and can promote the proliferation of cell tissues. After the bacterial cellulose membrane is covered with a biopolymer coating layer with antibacterial and degradable functions on the surface through an electrostatic spinning process, the obtained biological surgery patch can play a role of bacteria inhibition, infection prevention and tissue adhesion prevention, which is of great significance for the rapid recovery of patients after operation. Compared with other animal-derived base materials, the biological surgery patch described by the present invention uses bacterial cellulose membrane as a base material, and has the advantages of wide sources, low cost and safer clinical application.

In the follows, the method for preparing a biological surgery patch and a biological surgery patch prepared based on the method described by the present invention will be further described in detail in combination with specific Examples, and the technical solutions of the present invention include but are not limited to the following Examples.

Example 1

Step (1) preparation of a culture medium: 20 g of mannitol, 15 g of polyethylene glycol, 25 g of sucrose, 20 g of glucose, 0.5 mg of nicotinic acid, 5 g of yeast powder, 10 g of dry powder of corn steep liquor, 5 g of beef extract, 10 g of peptone, 2 g of KH_2PO_4 , 2 g of MgSO_4 , 8 g of anhydrous ethanol, 0.5 g of ammonium sulfate, 0.5 g of citric acid, 0.5 g of sodium carboxymethyl cellulose, 10 g of gluconic acid and 40 g of sodium gluconate were weighed and added into 1000 mL of purified water.

Step (2) fermentation: the microbial strain capable of producing cellulose membrane was activated to obtain an activated strain, the activated strain was transferred to a stainless steel tray for amplification culture to obtain a seed liquid, the obtained seed liquid was cultured in an incubator at a constant temperature of 37°C for 10 days, 2% mannitol and 5% polyethylene glycol were respectively added on the first day and the third day of the fermentation, and during the fermentation process, the pH of the system was maintained at 6.8 and the oxygen flux was 0.4 L/min, after the fermentation was completed, a bacterial cellulose membrane on the upper layer of the fermentation liquid was obtained, and the bacterial cellulose membrane was taken out for use.

Step (3) cleaning and pretreatment: the bacterial cellulose membrane obtained by fermentation was cleaned using water for injection until the pH value of the bacterial cellulose

membrane was 6, then the bacterial cellulose membrane was put into 1 L of 0.5 mol/L sodium hydroxide solution prepared by sterilized water for injection, soaked for 5 h, heated and boiled for 2 h, and then the resultant was taken out, soaked with sterilized water for injection and stirred for 50 min until the pH of a wrung-out solution was 6.0, then transferred to 1 L of 1 mol/L mixed solution of acetic acid and citric acid prepared with sterilized water for injection, wherein the mass ratio of acetic acid to citric acid was 4:1, and then the resultant was taken out after soaking for 5 h, soaked with water for injection and stirred with a glass rod for 3 min, and the washed water for injection was discarded.

Step (4) oxidation and purification treatment: the bacterial cellulose membrane obtained in step (3) was transferred into a 15% hydrogen peroxide solution, soaked and stirred for 30 min, taken out and loaded into a glass column, a vacuum pump was started for suction filtration, the bacterial cellulose membrane was continuously leached by using water for injection as liquid for leaching, and the leachate was detected, wherein the content of the obtained bacterial endotoxin was less than or equal to 0.03EU/ml, thus a purified bacterial cellulose membrane was obtained.

Step (5) freeze thawing and drying treatment: the purified bacterial cellulose membrane was frozen for 0.2 h under the environment of -45°C , and then taken out and placed at an environment of 5°C for 10 h for thawing, and the thawed water was discarded; the above operations was repeated twice. The bacterial cellulose membrane which was thawed for the last time was taken out for later use, and the thawed water was discarded.

Step (6) a compounding process: the bacterial cellulose membrane was put into a 0.5% glycerol solution for stirring at a rotating speed of 1000 rpm for 0.5 h, and then spread on a stainless steel mesh, transferred to a blast drying oven for drying at a temperature of 10°C for 24 h, after drying, the biological surgery patch base material was obtained and kept for later use.

Step (7) preparation of an electrostatic spinning solution: 10 mL of polylactic acid-dichloromethane solution with a concentration of 1.5 g/mL were prepared; and 10 mL of polycaprolactone-dichloromethane solution with a concentration of 1.5 g/mL were prepared for later use.

Step (8) electrostatic spinning: the biological surgery patch base material obtained in step (5) was fixed on a receiving device of an electrostatic spinning device, the polylactic acid-dichloromethane solution obtained in step (6) was first transferred into a syringe for electrostatic spinning to obtain a biological surgery patch intermediate product A1, wherein the

parameters for the electrostatic spinning were as follows: a voltage of 20 KV, and a receiving distance of 20 cm; then the polycaprolactone-dichloromethane solution was transferred into a syringe for electrostatic spinning with A1 to obtain a biological surgery patch intermediate product A2, wherein the parameters for the electrostatic spinning were as follows: a voltage of 20 KV, a receiving distance of 20 cm, and a mass ratio of the bacterial cellulose membrane to the polylactic acid or the polycaprolactone of 1.0:10.

Step (9) purifying of the intermediate product: the intermediate product of the biological surgery patch obtained in the step (7) was soaked into an ethanol solution with a concentration of 85% for 20 min, taken out, and transferred into water for injection for soaking for 20 min, and then subjected to freeze drying for forming.

Step (10) packaging: the dry formed sample was prepared into a specification with a thickness of 0.025 mm and a size of 5 mm×5 mm by using a membrane pressing machine, and then subjected to inner packing, outer packing, product sterilization (dosage of 35 KGy) and packaging, to obtain a final finished product.

Example 2

Step (1) preparation of a culture medium: 25 g of mannitol, 10 g of polyethylene glycol, 30 g of sucrose, 15 g of glucose, 0.8 mg of nicotinic acid, 6 g of yeast powder, 15 g of dry powder of corn steep liquor, 10 g of beef extract, 5 g of peptone, 1.5 g of KH_2PO_4 , 2.5 g of MgSO_4 , 10 g of anhydrous ethanol, 0.8 g of ammonium sulfate, 0.6 g of citric acid, 0.6 g of sodium carboxymethylcellulose, 10 g of gluconic acid and 50 g of sodium gluconate are weighed and added into 1000 mL of purified water.

Step (2) fermentation: the microbial strain capable of producing cellulose membrane was activated to obtain an activated strain, the activated strain was transferred to a stainless steel tray for amplification culture to obtain a seed liquid, the obtained seed liquid was cultured in an incubator at a constant temperature of 40°C for 6 days, 2% mannitol and 5% polyethylene glycol were respectively added on the first day and the third day of the fermentation, and during the fermentation process, the pH of the system was maintained at 3.8 and the oxygen flux was 3.5 L/min, after the fermentation was completed, a bacterial cellulose membrane on the upper layer of the fermentation liquid was obtained, and the bacterial cellulose membrane was taken out for use.

Step (3) cleaning and pretreatment: the bacterial cellulose membrane obtained by

fermentation was cleaned using water for injection until the pH value of the bacterial cellulose membrane was 6, then the bacterial cellulose membrane was put into 1 L of 0.75 mol/L sodium hydroxide solution prepared by sterilized water for injection, soaked for 5 h, heated and boiled for 2 h, and then the resultant was taken out, soaked with sterilized water for injection and stirred for 60 min until the pH of a wrung-out solution was 6.0, then transferred to 1 L of 1 mol/L mixed solution of acetic acid and citric acid prepared with sterilized water for injection, wherein the mass ratio of acetic acid to citric acid was 4:1, and then the resultant was taken out after soaking for 5 h, soaked with water for injection and stirred with a glass rod for 60 min, and the washed water for injection was discarded.

Step (4) oxidation and purification treatment: the bacterial cellulose membrane obtained in step (3) was transferred into a 30% hydrogen peroxide solution, soaked and stirred for 30 min, taken out and loaded into a glass column, a vacuum pump was started for suction filtration, the bacterial cellulose membrane was continuously leached by using water for injection as liquid for leaching, and the leachate was detected, the content of the obtained bacterial endotoxin was less than or equal to 0.03EU/ml, thus a purified bacterial cellulose membrane was obtained.

Step (5) freeze thawing and drying treatment: the purified bacterial cellulose membrane was frozen for 10 h under the environment of -1°C , and then taken out and placed at an environment of 35°C for 0.2 h for thawing, and the thawed water was discarded; the above operations was repeated for 9 times. The bacterial cellulose membrane which was thawed for the last time was taken out for later use, and the thawed water was discarded.

Step (6) a compounding process: the bacterial cellulose membrane was put into a 3% glycerol solution for stirring at a rotating speed of 20 rpm for 2 h, and then spread on a stainless steel mesh, transferred to a blast drying oven for drying at a temperature of 50°C for 12 h, after drying, the biological surgery patch base material was obtained and kept for later use.

Step (7) preparation of an electrostatic spinning solution: 10 mL of polylactic acid-dichloromethane solution with a concentration of 4.5% were prepared; and 10 mL of polycaprolactone-dichloromethane solution with a concentration of 4.5% were prepared for later use.

Step (8) electrostatic spinning: the biological surgery patch base material obtained in step (5) was fixed on a receiving device of an electrostatic spinning device, the polylactic acid-dichloromethane solution obtained in step (6) was first transferred into a syringe for

electrostatic spinning to obtain a biological surgery patch intermediate product A1, wherein the parameters for the electrostatic spinning were as follows: a voltage of 10 KV, and a receiving distance of 5 cm; then the polycaprolactone-dichloromethane solution was transferred into a syringe for electrostatic spinning with A1 to obtain a biological surgery patch intermediate product A2, wherein the parameters for the electrostatic spinning were as follows: a voltage of 30 KV, a receiving distance of 30 cm, and a mass ratio of the bacterial cellulose membrane to the polylactic acid or the polycaprolactone of 0.1:10.

Step (9) purifying of the intermediate product: the intermediate product of the biological surgery patch obtained in step (7) was soaked into an ethanol solution with a concentration of 50% for 60 min, taken out, and transferred into water for injection for soaking for 10 min, and then subjected to freeze drying for forming.

Step (10) packaging: the dry formed sample was prepared into a specification with a thickness of 0.025 mm and a size of 50 mm×50 mm by using a membrane pressing machine, and then subjected to inner packing, outer packing, product sterilization (dosage of 25 KGy) and packaging, to obtain a final finished product.

Example 3

Step (1) preparation of a culture medium: 22 g of mannitol, 13 g of polyethylene glycol, 28 g of sucrose, 18 g of glucose, 0.65 mg of nicotinic acid, 6 g of yeast powder, 12 g of dry powder of corn steep liquor, 8 g of beef extract, 8 g of peptone, 2.0 g of KH_2PO_4 , 2.2 g of MgSO_4 , 9 g of anhydrous ethanol, 0.7 g of ammonium sulfate, 0.6 g of citric acid, 0.6 g of sodium carboxymethylcellulose, 15 g of gluconic acid and 30 g of sodium gluconate were weighed and added into 1000 mL of purified water.

Step (2) fermentation: the microbial strain capable of producing cellulose membrane was activated to obtain an activated strain, the activated strain was transferred to a stainless steel tray for amplification culture to obtain a seed liquid, the obtained seed liquid was cultured in an incubator at a constant temperature of 25°C for 11 days, 1% mannitol and 3% polyethylene glycol were respectively added on the first day and the third day of the fermentation, and during the fermentation process, the pH of the system was maintained at 7 and the oxygen flux was 8 L/min, after the fermentation was completed, a bacterial cellulose membrane on the upper layer of the fermentation liquid was obtained, and the bacterial cellulose membrane was taken out for use.

Step (3) cleaning and pretreatment: the bacterial cellulose membrane obtained by fermentation was cleaned using water for injection until the pH value of the bacterial cellulose membrane was 6, then the bacterial cellulose membrane was put into 1 L of 1.0 mol/L sodium hydroxide solution prepared by sterilized water for injection, soaked for 5 h, heated and boiled for 2 h, and then the resultant was taken out, soaked with sterilized water for injection and stirred for 60 min until the pH of a wrung-out solution was 6.0, then transferred to 1 L of 1 mol/L mixed solution of acetic acid and citric acid prepared with sterilized water for injection, wherein the mass ratio of acetic acid to citric acid was 4:1, and then the resultant was taken out after soaking for 5 h, soaked with water for injection and stirred with a glass rod for 60 min, and the washed water for injection was discarded.

Step (4) oxidation and purification treatment: the bacterial cellulose membrane obtained in the step (3) was transferred into a 30% hydrogen peroxide solution, soaked and stirred for 30min, taken out and loaded into a glass column, a vacuum pump was started for suction filtration, the bacterial cellulose membrane was continuously leached by using water for injection as liquid for leaching, and the leachate was detected, wherein the content of the obtained bacterial endotoxin was less than or equal to 0.03EU/ml, thus a purified bacterial cellulose membrane was obtained.

Step (5) freeze thawing and drying treatment: the purified bacterial cellulose membrane was frozen for 10 h under the environment of -1°C , and then taken out and placed at an environment of 5°C for 10 h for thawing, and the thawed water was discarded; the above operations was repeated twice. The bacterial cellulose membrane which was thawed for the last time was taken out for later use, and the thawed water was discarded.

Step (6) a compounding process: the bacterial cellulose membrane was put into a 1.5% glycerol solution for stirring at a rotating speed of 500 rpm for 1 h, and then spread on a stainless steel mesh, transferred to a blast drying oven for drying at a temperature of 99°C for 2 h, after drying, the biological surgery patch base material was obtained and kept for later use.

Step (7) preparation of an electrostatic spinning solution: 10 mL of polylactic acid-dichloromethane solution with a concentration of 5% were prepared; and 10 mL of polycaprolactone-dichloromethane solution with a concentration of 5% were prepared for later use.

Step (8) electrostatic spinning: the biological surgery patch base material obtained in step (5) was fixed on a receiving device of an electrostatic spinning device, the polylactic

acid-dichloromethane solution obtained in step (6) was first transferred into a syringe for electrostatic spinning to obtain a biological surgery patch intermediate product A1, wherein the parameters for the electrostatic spinning parameter were as follows: a voltage of 30 KV, and a receiving distance of 30 cm; then the polycaprolactone-dichloromethane solution was transferred into a syringe for electrostatic spinning with A1 to obtain a biological surgery patch intermediate product A2, wherein the parameters for the electrostatic spinning were as follows: a voltage of 10 KV, a receiving distance of 5 cm, and a mass ratio of the bacterial cellulose membrane to the polylactic acid or the polycaprolactone of 3.0: 10.

Step (9) purifying of the intermediate product: the intermediate product of the biological surgery patch obtained in the step (7) was soaked into an ethanol solution with a concentration of 99% for 10 min, taken out, and transferred into water for injection for soaking for 60 min, and then subjected to freeze drying for forming.

Step (10) packaging: the dry formed sample was prepared into a specification with a thickness of 0.010 mm and a size of 5 mm×50 mm by using a membrane pressing machine, and then subjected to inner packing, outer packing, product sterilization (dosage of 5 KGy) and packaging, to obtain a final finished product.

Example 4

Step (1) preparation of a culture medium: 25 g of mannitol, 10 g of polyethylene glycol, 30 g of sucrose, 19 g of glucose, 0.8 mg of nicotinic acid, 6 g of yeast extract, 13 g of dry powder of corn steep liquor, 10 g of beef extract, 5 g of peptone, 2.5 g of KH_2PO_4 , 2.0 g of MgSO_4 , 8 g of anhydrous ethanol, 0.5 g of ammonium sulfate, 0.6 g of citric acid, 0.6 g of sodium carboxymethylcellulose, 10 g of gluconic acid and 35 g of sodium gluconate were weighed and added into 1000 mL of purified water.

Step (2) fermentation: the microbial strain capable of producing cellulose membrane was activated to obtain an activated strain, the activated strain was transferred to a stainless steel tray for amplification culture to obtain a seed liquid, the obtained seed liquid was cultured in an incubator at a constant temperature of 32°C for 10 days, 1.5% mannitol and 5% polyethylene glycol were respectively added on the first day and the third day of the fermentation, and during the fermentation process, the pH of the system was maintained at 5.2 and the oxygen flux was 0.3 L/min, after the fermentation was completed, a bacterial cellulose membrane on the upper layer of the fermentation liquid was obtained, and the bacterial cellulose membrane was taken

out for use.

Step (3) cleaning and pretreatment: the bacterial cellulose membrane obtained by fermentation was cleaned using water for injection until the pH value of the bacterial cellulose membrane was 6, then the bacterial cellulose membrane was put into 1 L of 1.0 mol/L sodium hydroxide solution prepared by sterilized water for injection, soaked for 3 h, heated and boiled for 2 h, and then the resultant was taken out, soaked with sterilized water for injection and stirred for 60 min until the pH of a wrung-out solution was 6.0, then transferred to 1 L of 1 mol/L mixed solution of acetic acid and citric acid prepared with sterilized water for injection, wherein the mass ratio of acetic acid to citric acid was 4:1, and then the resultant was taken out after soaking for 5 h, soaked with water for injection and stirred with a glass rod for 30 min, and the washed water for injection was discarded.

Step (4) oxidation and purification treatment: the bacterial cellulose membrane obtained in the step (3) was transferred into a 50% hydrogen peroxide solution, soaked and stirred for 30 min, taken out and loaded into a glass column, a vacuum pump was started for suction filtration, the bacterial cellulose membrane was continuously leached by using water for injection as liquid for leaching, and the leachate was detected, wherein the content of the obtained bacterial endotoxin was less than or equal to 0.03EU/ml, thus a purified bacterial cellulose membrane was obtained.

Step (5) freeze thawing and drying treatment: the purified bacterial cellulose membrane was frozen for 6 h under the environment of -25°C, and then taken out and placed at an environment of 15°C for 5 h for thawing, and the thawed water was discarded; the above operations was repeated twice. The bacterial cellulose membrane which was thawed for the last time was taken out for later use, and the thawed water was discarded.

Step (6) a compounding process: the bacterial cellulose membrane was put into a 2.5% glycerol solution for stirring at a rotating speed of 800 rpm for 0.75 h, and then spread on a stainless steel mesh, transferred to a blast drying oven for drying at a temperature of 65°C for 5 h, after drying, and the biological surgery patch base material was obtained and kept for later use.

Step (7) preparation of an electrostatic spinning solution: 10 mL of polylactic acid-dichloromethane solution with a concentration of 0.5% were prepared; and 10 mL of polycaprolactone-dichloromethane solution with a concentration of 0.5% were prepared for later use.

Step (8) electrostatic spinning: the biological surgery patch base material obtained in step (5)

was fixed on a receiving device of an electrostatic spinning device, the polylactic acid-dichloromethane solution obtained in step (6) was first transferred into a syringe for electrostatic spinning to obtain a biological surgery patch intermediate product A1, wherein the parameters for the electrostatic spinning were as follows: a voltage of 20 KV, and a receiving distance of 20 cm; then the polycaprolactone-dichloromethane solution was transferred into a syringe for electrostatic spinning with A1 to obtain a biological surgery patch intermediate product A2, wherein the parameters for the electrostatic spinning were as follows: a voltage of 15 KV, a receiving distance of 15 cm, and the mass ratio of the bacterial cellulose membrane to the polylactic acid or the polycaprolactone of 1.0:10.

Step (9) purifying of the intermediate product: the intermediate product of the biological surgery patch obtained in the step (7) was soaked into an ethanol solution with a concentration of 75% for 40 min, taken out, and transferred into water for injection for soaking for 40 min, and then subjected to freeze drying for forming.

Step (10) packaging: the dry formed sample was prepared into a specification with a thickness of 0.04 mm and a size of 50 mm×100 mm by using a membrane pressing machine, and then subjected to inner packing, outer packing, product sterilization (dosage of 50 KGy) and packaging, to obtain a final finished product.

The advantageous effects are shown in table 1.

Table 1: Parameters of the biological surgery patch

No	Preparation parameters	Dry film thickness (mm)	Tensile strength (MPa)	Elongation at break (%)	Porosity (%)	Bursting strength (KPa)	Stitching strength (N)
01	See Example 1	0.025	72.173	20.1	92.4	60.4	18.6
02	See Example 2	0.025	68.315	19.8	93.6	61.2	18.3
03	See Example 3	0.010	60.062	20.5	90.8	56.8	15.5
04	See Example 4	0.040	89.389	18.0	90.7	92.6	26.0

The above description are only preferred embodiments of the present invention, and it

should be noted that, a person skilled in the art can make various improvements and modifications without departing from the technical principles of the present invention, and these improvements and modifications should also be regarded as being within the protection scope of the present invention.

Industrial applicability

The present invention provides a preparation method for a biological surgery patch, a biological surgery patch prepared by the method and an application thereof. A base material of the preparation method is a bacterial cellulose membrane prepared by biological fermentation: the bacterial cellulose membrane prepared by biological fermentation is subjected to purification, oxidation, freeze thawing, composite process processing, electrostatic spinning, freeze drying and membrane pressing to obtain the biological surgery patch. The biological surgery patch is good in biocompatibility, mechanical properties, sewing performance and clinical application convenience and capable of satisfying the basic requirements of surgery patch materials, and has good economic value and application prospect.

What is claimed is:

1. A preparation method for a biological surgery patch, comprising the following steps:

(1) fermenting culturing a strain to obtain a bacterial cellulose membrane; adding biopolymer materials during the fermenting culturing process; the biopolymer materials include one or more of polylactic acid, polyamide, polyester, glycerol, polylactide, polycaprolactone, polyvinyl chloride and polyethylene;

(2) soaking the bacterial cellulose membrane obtained in step (1) in an inorganic base solution and an acid solution sequentially to obtain a purified bacterial cellulose membrane;

(3) immersing the purified bacterial cellulose membrane obtained in step (2) into an oxidant solution for oxidation to obtain an oxidized bacterial cellulose membrane;

(4) subjecting the oxidized bacterial cellulose membrane obtained in step (3) to freeze-thawing to obtain a freeze-thawed bacterial cellulose membrane;

(5) placing the freeze-thawed bacterial cellulose membrane obtained in step (4) into a glycerol aqueous solution, stirring and drying to obtain a composite processed bacterial cellulose membrane;

(6) performing electrostatic spinning on the composite processed bacterial cellulose membrane obtained in step (5) to obtain an intermediate product of a biological surgery patch; the electrostatic spinning solution includes a solution of one or more of polylactic acid, polycaprolactone, chitosan, polyacrylonitrile and polycarbonate; the solvent of the solution is one or more of dichloromethane, trichloromethane, tetrahydrofuran, N,N-dimethylformamide, N,N-dimethylacetamide, dimethyl sulfoxide, diethyl ether, n-hexane and petroleum ether; and

(7) subjecting the intermediate product of a biological surgery patch obtained in step (6) to freeze drying and membrane pressing to obtain the biological surgery patch;

wherein, each 1000 mL of the medium for the fermenting culturing in step (1) comprises: 20 g to 25 g of mannitol, 10 g to 15 g of polyethylene glycol, 25 g to 30 g of sucrose, 15 g to 20 g of glucose, 0.5 mg to 0.8 mg of nicotinic acid, 5 g to 6 g of yeast powder, 10 g to 15 g of dry powder of corn steep liquor, 5 g to 10 g of beef extract, 5 g to 10 g of peptone, 1.5 g to 2.5 g of KH_2PO_4 , 2.0 g to 2.5 g of MgSO_4 , 8 g to 10 g of anhydrous ethanol, 0.5 g to 0.8 g of ammonium sulfate, 0.5 g to 0.6 g of citric acid, 0.5 g to 0.6 g of sodium carboxymethylcellulose and 40 g to 50 g of a mixture of gluconic acid and sodium gluconate; and the mass ratio of the gluconic acid to the sodium gluconate is 1: (1 to 5).

2. The preparation method according to claim 1, wherein, the strain of step (1) comprises one or more of *Acetobacter*, *Rhizobium*, *Gluconobacter*, *Acetobacter xylinum*, *Sarcina*, *Pseudomonas*, *Achromobacter*, *Alcaligenes*, *Aerobacter*, *Azotobacter*, and *Agrobacterium*.

3. The preparation method according to claim 1, wherein, the fermenting culturing in step (1) is performed at 20°C to 40°C with an oxygen flux of 0.01 L/min to 10 L/min and a pH value of 3 to 9 for 6 days to 14 days.

4. The preparation method according to claim 1, wherein, when the biopolymer materials in step (1) include a plurality of types, the biopolymer materials are added one by one in the order of polylactic acid, polyamide, polyester, glycerol, polylactide, polycaprolactone, polyvinyl chloride, and polyethylene.

5. The preparation method according to claim 1, wherein, the oxidant in step (3) is an aqueous solution comprising one or more of hydrogen peroxide, sodium hypochlorite, sodium peroxide, potassium peroxide, peracetic acid, sodium percarbonate, sodium perborate and potassium perborate.

6. The preparation method according to claim 5, wherein, the mass fractions of the hydrogen peroxide, sodium hypochlorite, sodium peroxide, potassium peroxide, peroxyacetic acid, sodium percarbonate, sodium perborate and potassium perborate in water are 1% to 50%, respectively.

7. The preparation method according to claim 1, wherein, in step (4), the freeze-thawing is carried out as follows: freezing the oxidized bacterial cellulose membrane obtained in step (3) at -45°C to -1°C for 0.2 to 10 h, taking out and thawing under the environment of 5°C to 35°C for 0.2 to 10 hours, and discarding the thawed water; repeating the above freezing and thawing operations for 2 to 9 times.

8. The preparation method according to claim 1, wherein, the mass concentrations of polylactic acid, polycaprolactone, chitosan, polyacrylonitrile or polycarbonate in the electrostatic spinning solution in step (6) are 0.01 g/mL to 5 g/mL, respectively.

9. The preparation method according to claim 1, wherein, the mass ratios of the composite processed bacterial cellulose membrane in step (6) to polylactic acid, polyacrylonitrile, polycarbonate, chitosan or polycaprolactone are (0.1 to 3):10, respectively.

10. The biological surgery patch prepared by the preparation method according to any one of claims 1 to 9, wherein, the biological surgery patch is composed of bacterial cellulose

and has a nano-network structure.

11. The biological surgery patch according to claim 10, wherein, the bacterial cellulose has a Young's modulus of 10 to 1000 GPa and a tensile strength of 0.1 to 100 Gpa.

12. The biological surgery patch according to claim 10, wherein, the diameter of the fiber in the nano-network structure is 10 to 200 nm.

13. The biological surgery patch according to claim 10 or 12, wherein, the porosity of the nano-network structure is 60% to 99%; and the pore size of the nano-network structure is 10 to 500 nm.

14. Use of the biological surgery patch according to any one of claims 10 to 13 for the preparation of a repair material for medical use.