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(54) NONWOVEN FABRIC, AND METHOD FOR PRODUCING SAME

(57) An object of the present invention is to provide a nonwoven fabric that makes it difficult to diffuse liquid in a plane direction and can absorb and transmit liquid in a spot manner, that is, a nonwoven fabric excellent in so-called spot absorbability. The nonwoven fabric according to the present invention is formed by mixing an artificial protein fiber and a hydrophobic synthetic fiber.

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Description

Technical Field

[0001] The present invention relates to a nonwoven fabric excellent in absorption characteristics of a liquid such as a body fluid or water, and a method for producing the nonwoven fabric, and particularly relates to a nonwoven fabric that makes it difficult to diffuse liquid in a plane direction and can absorb and transmit liquid in a spot manner, that is, a nonwoven fabric excellent in liquid absorption characteristics, and a method for producing the nonwoven fabric.

Background Art

[0002] Conventionally, a nonwoven fabric having a good touch is used as a surface material of a sanitary material such as a sanitary napkin or a disposable diaper. As the nonwoven fabric used as a surface material of a sanitary material, a nonwoven fabric that easily absorbs and transmits a body fluid and makes it difficult to diffuse liquid in a plane direction, that is, a nonwoven fabric excellent in so-called spot absorbability is required. Various studies have been made in order to improve spot absorbability, and for example, a nonwoven fabric obtained by mixing a short fiber, a long fiber, and a lump-shaped particle has been proposed (Patent Literature 1).

Citation List

Patent Literature

[0003] Patent Literature 1: JP 2008-125602 A

Summary of Invention

Technical Problem

[0004] An object of the present invention is to provide a nonwoven fabric excellent in spot absorbability and a method capable of advantageously producing such a nonwoven fabric in a simpler process.

Solution to Problem

[0005] As a result of various studies to solve such problems, the present inventors have found that excellent spot absorbability can be achieved in a nonwoven fabric obtained by mixing (intermingling) an artificial protein fiber containing an artificial fibroin and a hydrophobic synthetic fiber.

[0006] That is, the present invention has been completed based on the above finding, and relates to a nonwoven fabric having a region formed by mixing an artificial protein fiber containing an artificial fibroin and a hydrophobic synthetic fiber and having excellent liquid absorption characteristics.

[0007] The present invention also relates to a method for producing a nonwoven fabric, including a step of mixing an artificial protein fiber containing an artificial fibroin and a hydrophobic synthetic fiber.

[0008] Hereinafter, an embodiment of the present invention will be described in detail. Note that the present invention is not limited to the following embodiment.

[0009] A nonwoven fabric according to the present embodiment is formed by mixing (intermingling) an artificial protein fiber containing an artificial fibroin and a hydrophobic synthetic fiber. In the artificial protein fiber constituting such a nonwoven fabric, all of the artificial proteins are preferably artificial fibroins, but natural or artificial proteins other than the artificial fibroins and various additives may be contained as long as an effect exhibited in the nonwoven fabric of the present invention is not inhibited. In addition, the artificial protein fiber may be a short fiber or a long fiber. Furthermore, the artificial protein fiber may be crimped or does not have to be crimped as long as the artificial protein fiber can form a nonwoven fabric.

[0010] The term "fibroin" used in the present specification means a protein molecule produced by an insect such as silkworm or a spider. A fibroin may mean a fibrous one in which fibrils each constituted by protein molecules are bundled, but from this viewpoint, the "fibroin" used in the present specification means a fibroin molecule, that is, a protein molecule constituting the fibroin. Note that the protein molecule may be simply referred to as a protein.

[0011] An artificial fibroin includes a modified (recombinant) fibroin and a synthetic fibroin. That is, the "artificial fibroin" used in the present specification means an artificially produced protein having an amino acid sequence equivalent or similar to that of a protein produced by an insect such as a silkworm or a spider. The artificial fibroin may be a fibroin having a domain sequence different from an amino acid sequence of a naturally derived fibroin, or may be a protein

having a domain sequence identical to an amino acid sequence of a naturally derived fibroin. In other words, such an artificial fibroin may be artificially produced using an amino acid sequence of a naturally derived fibroin as it is, may be obtained by modifying an amino acid sequence of a naturally derived fibroin based on the amino acid sequence (such as a fibroin having a modified amino acid sequence obtained by modifying a cloned gene sequence of a naturally derived fibroin), or may be an artificially designed and synthesized one independently of a naturally derived fibroin (such as a fibroin having a desired amino acid sequence obtained by chemically synthesizing a nucleic acid encoding a designed amino acid sequence).

[0012] As the artificial fibroin contained in the artificial protein fiber as a constituent material of the nonwoven fabric according to the present embodiment, a modified fibroin is preferably used, and among the modified fibroins, a modified fibroin having an amino acid sequence derived from spider silk, so-called a modified spider silk fibroin is more preferably used.

[0013] Examples of such a modified fibroin include a modified fibroin described in WO 2020/067546 A (a modified spider silk fibroin). That is, preferable examples of the modified fibroin include a modified fibroin derived from a major dragline silk protein produced in a major ampullate gland of a spider (first modified fibroin), a modified fibroin having a domain sequence in which the content of glycine residues is reduced (second modified fibroin), a modified fibroin having a domain sequence in which the content of an (A)n motif is reduced (third modified fibroin), a modified fibroin in which the content of glycine residues and the content of the (A)n motif are reduced (fourth modified fibroin), a modified fibroin having a domain sequence including a region locally having a high hydropathy index (fifth modified fibroin), and a modified fibroin having a domain sequence in which the content of glutamine residues is reduced (sixth modified fibroin).

First modified fibroin

[0014] Examples of the first modified fibroin include a protein having a domain sequence represented by formula 1: [(A)n motif-REP]m. In formula 1, the (A)n motif represents an amino acid sequence consisting of 3 to 20 amino acid residues, the number of alanine residues with respect to the total number of amino acid residues in the (A)n motif is 83% or more, and the total number of glycine residues, serine residues, and alanine residues contained in the amino acid sequence represented by formula 1: [(A)n motif-REP]m is 40% or more with respect to the total number of amino acid residues. "REP" represents an amino acid sequence consisting of 10 to 200 amino acid residues, m represents an integer of 8 to 300. The plurality of (A)n motifs may be identical or different amino acid sequences. The plurality of REPs may be identical or different amino acid sequences.

[0015] The first modified fibroin may be a polypeptide including an amino acid sequence unit represented by formula 1: [(A)n motif-REP]m, and including a C-terminal sequence which is an amino acid sequence set forth in any one of SEQ ID NOs: 1 to 3 or a C-terminal sequence which is an amino acid sequence having 90% or more homology with the amino acid sequence set forth in any one of SEQ ID NOs: 1 to 3. Specific examples of such a first modified fibroin include a modified fibroin having the amino acid sequence set forth in SEQ ID NO: 4 (recombinant spider silk protein ADF3KaiLargeNRSH1) or an amino acid sequence having a sequence identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 4. The sequence identity is preferably 95% or more. In addition, the first modified fibroin may consist of the amino acid sequence set forth in SEQ ID NO: 4. Note that the amino acid sequence set forth in SEQ ID NO: 4 is obtained by causing a mutation such that, in an amino acid sequence of ADF3 having an N-terminal to which an amino acid sequence (SEQ ID NO: 5) consisting of a start codon, a His10-tag, and an HRV3C protease (human rhinovirus 3C protease) recognition site is added, the first to thirteenth repeat regions are increased to be nearly doubled, and translation ends at the 1154th amino acid residue. The C-terminal amino acid sequence of the amino acid sequence set forth in SEQ ID NO: 4 is identical to the amino acid sequence set forth in SEQ ID NO: 3.

Second modified fibroin

[0016] The second modified fibroin has a domain sequence represented by formula 1: [(A)n motif-REP]m, and the domain sequence has an amino acid sequence in which the content of glycine residues is reduced, corresponding to an amino acid sequence in which at least one or more glycine residues in REP are replaced with other amino acid residues, as compared with a naturally derived fibroin.

[0017] More specific examples of the second modified fibroin include a modified fibroin having the amino acid sequence set forth in SEQ ID NO: 6, 7, 8, 9, 10, 12, 13, 14, 15, or 16 or an amino acid sequence having a sequence identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 6, 7, 8, 9, 10, 12, 13, 14, 15, or 16. The amino acid sequences set forth in SEQ ID NOs: 12, 13, 14, 15, and 16 are obtained by adding the amino acid sequence set forth in SEQ ID NO: 11 (amino acid sequence having a His tag sequence and a hinge sequence) to N-terminals of the amino acid sequences set forth in SEQ ID NOs: 6, 7, 8, 9, and 10, respectively.

Third modified fibroin

[0018] The third modified fibroin has a domain sequence represented by formula 1: [(A)n motif-REP]m, and the domain sequence has an amino acid sequence in which the content of the (A)n motif is reduced, as compared with a naturally derived fibroin. It can be said that the domain sequence of the third modified fibroin has an amino acid sequence corresponding to an amino acid sequence in which at least one or more (A)n motifs are deleted, as compared with a naturally derived fibroin.

[0019] More specific examples of the third modified fibroin include a modified fibroin having the amino acid sequence set forth in SEQ ID NO: 7, 8, 9, 17, 13, 14, 15, or 18 or an amino acid sequence having a sequence identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 7, 8, 9, 17, 13, 14, 15, or 18. The amino acid sequences set forth in SEQ ID NOs: 13, 14, 15, and 18 are obtained by adding the amino acid sequence set forth in SEQ ID NO: 11 to N-terminals of the amino acid sequences set forth in SEQ ID NO: 7, 8, 9, and 17, respectively.

Fourth modified fibroin

[0020] The fourth modified fibroin has a domain sequence represented by formula 1: [(A)n motif-REP]m, and has an amino acid sequence in which the content of the (A)n motif is reduced and the content of glycine residues is reduced, as compared with a naturally derived fibroin. It can be said that the domain sequence of the fourth modified fibroin has an amino acid sequence corresponding to an amino acid sequence in which at least one or more (A)n motifs are deleted and at least one or more glycine residues in REP are replaced with other amino acid residues, as compared with a naturally derived fibroin. That is, the fourth modified fibroin is a modified fibroin having characteristics of both the second modified fibroin and the third modified fibroin described above. Specific aspects thereof and the like are as in the descriptions for the second modified fibroin and the third modified fibroin.

[0021] More specific examples of the fourth modified fibroin include a modified fibroin having the amino acid sequence set forth in SEQ ID NO: 7, 8, 9, 13, 14, or 15 or an amino acid sequence having a sequence identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 7, 8, 9, 13, 14, or 15.

Fifth modified fibroin

[0022] The fifth modified fibroin has a domain sequence represented by formula 1: [(A)n motif-REP]m, and the domain sequence has an amino acid sequence including a region locally having a high hydropathy index, corresponding to an amino acid sequence in which one or more amino acid residues in REP are replaced with amino acid residues having a high hydropathy index, and/or an amino acid sequence in which one or more amino acid residues having a high hydropathy index are inserted into REP, as compared with a naturally derived fibroin. The amino acid residue having a high hydropathy index is preferably selected from the group consisting of isoleucine (I), valine (V), leucine (L), phenylalanine (F), cysteine (C), methionine (M), and alanine (A), and is more preferably valine (V), leucine (L), or isoleucine (I).

[0023] As the hydropathy index of an amino acid residue, a known index (Hydropathy index: Kyte J & Doolittle R (1982) "A simple method for displaying the hydropathic character of a protein", J. Mol. Biol., 157, pp. 105-132) is used. Specifically, the hydropathy indices (hereinafter also referred to as "HI") of amino acids are as represented in Table 1 below.

[Table 1]

Amino acid	HI	Amino acid	HI
Isoleucine (Ile)	4.5	Tryptophan (Trp)	-0.9
Valine (Val)	4.2	Tyrosine (Tyr)	-1.3
Leucine (Leu)	3.8	Proline (Pro)	-1.6
Phenylalanine (Phe)	2.8	Histidine (His)	-3.2
Cysteine (Cys)	2.5	Asparagine (Asn)	-3.5
Methionine (Met)	1.9	Aspartic Acid (Asp)	-3.5
Alanine (Ala)	1.8	Glutamine (Gln)	-3.5
Glycine (Gly)	-0.4	Glutamic Acid (Glu)	-3.5
Threonine (Thr)	-0.7	Lysine (Lys)	-3.9
Serine (Ser)	-0.8	Arginine (Arg)	-4.5

[0024] More specific examples of the fifth modified fibroin include a modified fibroin having the amino acid sequence set forth in SEQ ID NO: 19, 20, 21, 22, 23, or 24 or an amino acid sequence having a sequence identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 19, 20, 21, 22, 23, or 24. The amino acid sequences set forth in SEQ ID NOs: 22, 23, and 24 are obtained by adding the amino acid sequence set forth in SEQ ID NO: 11 to N-terminals of the amino acid sequences set forth in SEQ ID NO: 19, 20, and 21, respectively.

Sixth modified fibroin

[0025] The sixth modified fibroin has a domain sequence represented by formula 1: [(A)n motif-REP]m, and has an amino acid sequence in which the content of glutamine residues is reduced, as compared with a naturally derived fibroin. The domain sequence of the sixth modified fibroin may have an amino acid sequence corresponding to an amino acid sequence in which one or more glutamine residues in REP are deleted or replaced with other amino acid residues, as compared with a naturally derived fibroin. As the "other amino acid residues", any amino acid residue other than the glutamine residue can be used, but an amino acid residue having a higher hydropathy index than the glutamine residue is preferable. The hydropathy indices of amino acid residues are presented in Table 1 above.

[0026] More specific examples of the sixth modified fibroin include a modified fibroin having the amino acid sequence set forth in SEQ ID NO: 25, 26, 27, 28, 29, 30, 31, 32, 41, 42, 33, 34, 35, 36, 37, 38, 39, 40, 43, or 44 or an amino acid sequence having a sequence identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 25, 26, 27, 28, 29, 30, 31, 32, 41, 42, 33, 34, 35, 36, 37, 38, 39, 40, 43, or 44. The amino acid sequences set forth in SEQ ID NOs: 33 to 40, 43, and 44 are obtained by adding the amino acid sequence set forth in SEQ ID NO: 11 to N-terminals of the amino acid sequences set forth in SEQ ID NO: 25 to 32, 41, and 42, respectively.

[0027] The modified fibroin may have at least two characteristics among the characteristics of the first modified fibroin, the second modified fibroin, the third modified fibroin, the fourth modified fibroin, the fifth modified fibroin, and the sixth modified fibroin.

[0028] The modified fibroin may be a hydrophilic modified fibroin or a hydrophobic modified fibroin. In the present specification, the "hydrophilic modified fibroin" is a modified fibroin of which a value (average HI) calculated by determining the sum of hydropathy indices (HIs) of all amino acid residues constituting the modified fibroin and then dividing the sum by the total number of amino acid residues is 0 or less. The hydropathy indices are presented in Table 1 above. In addition, the "hydrophobic modified fibroin" is a modified fibroin of which the average HI is more than 0. Examples of the hydrophilic modified fibroin include those included in the definitions of the first to fourth modified fibroins described above, and examples of the hydrophobic modified fibroin include those included in the definitions of the fifth and sixth modified fibroins described above.

[0029] The modified fibroin contained in the artificial protein fiber of the nonwoven fabric according to the present embodiment may be a modified fibroin (a hydrophilic modified fibroin or a hydrophobic modified fibroin) having an average HI of -1.0 or more, a modified fibroin having an average HI of -0.8 or more (a hydrophilic modified fibroin or a hydrophobic modified fibroin), or a modified fibroin (a hydrophobic modified fibroin) having an average HI of more than 0. When the modified fibroin contained in the artificial protein fiber has an average HI of -1.0 or more or -0.8 or more, more sufficient water absorbability may be obtained. When the modified fibroin contained in the artificial protein fiber has an average HI of more than 0, diffusion in a plane direction may be more effectively suppressed.

[0030] As a method for forming an artificial protein fiber using an artificial fibroin, it is only required to adopt a known method (for example, a dry-wet spinning method, a wet spinning method, or a wet spinning method) such as the method described in WO 2020/067546 A. That is, it is only required to discharge a spinning solution in which an artificial fibroin is dissolved from a nozzle to form a fiber, thereby obtaining an artificial protein fiber. In addition, an artificial fibroin may be mixed with another protein, a high molecular weight polymer, various additives, and the like, and a spinning solution in which the resulting mixture is dissolved may be discharged from a nozzle to form a fiber, thereby obtaining an artificial protein fiber. The artificial protein fiber containing an artificial fibroin may be a short fiber or a long fiber. In addition, the artificial protein fiber containing an artificial fibroin may have any fineness, and for example, has a fineness of about 1 to 10 dtex.

[0031] The synthetic fiber constituting the nonwoven fabric according to the present embodiment together with the artificial protein fiber is hydrophobic. When a hydrophilic synthetic fiber is adopted, spot absorbability of an obtained nonwoven fabric decreases, which is not preferable. The hydrophobic synthetic fiber preferably has an official moisture regain of less than 2%. When the official moisture regain is more than 2%, the spot absorbability of an obtained nonwoven fabric tends to decrease. The official moisture regain of the hydrophobic synthetic fiber is preferably 1.5% or less, more preferably 1.0% or less, and still more preferably 0.5% or less. The lower the official moisture regain, the higher the spot absorbability of the nonwoven fabric may be. Specific examples of the hydrophobic synthetic fiber include a polyester fiber such as a polyethylene terephthalate fiber, a polyolefin fiber such as a polypropylene fiber, a biodegradable fiber such as a polylactic acid fiber, and a stretchable fiber such as a polyurethane fiber. These fibers can be used singly or in combination thereof as appropriate. The hydrophobic synthetic fiber may also be a short fiber or a long fiber. The

hydrophobic synthetic fiber may also have any fineness, and for example, has a fineness of about 1 to 10 dtex.

[0032] The nonwoven fabric according to the present invention has a region formed by mixing an artificial protein fiber and a hydrophobic synthetic fiber. Spot absorbability is exhibited in this region. Such a region may be the entire region of the nonwoven fabric or a partial region thereof. In particular, when the artificial protein fiber and the hydrophobic synthetic fiber are mixed in the entire region of the nonwoven fabric, spot absorbability is exhibited in any region of the nonwoven fabric, which is preferable. Note that a reason why excellent spot absorbability can be obtained in such a nonwoven fabric is not clear. However, it is considered that this is because sufficient water absorbability of the artificial protein fiber can be ensured by inclusion of the artificial protein fiber, and development of a capillary phenomenon occurring between fibers is suppressed by mixing of the artificial protein fiber and the hydrophobic synthetic fiber, thereby suppressing diffusion of water in a plane direction.

[0033] Note that in the nonwoven fabric according to the present embodiment, spot absorbability is more favorably exhibited when the degree of mixing of the artificial protein fiber and the hydrophobic synthetic fiber is relatively uniform. Furthermore, a region where the artificial protein fiber and the hydrophobic synthetic fiber are mixed may be formed in a part of the nonwoven fabric, and for example, may be formed in a stripe shape in a longitudinal direction of the nonwoven fabric or a width direction thereof. A region other than the region where the artificial protein fiber and the hydrophobic synthetic fiber are mixed may be constituted only by an artificial protein fiber, may be constituted only by a hydrophobic synthetic fiber, or may be constituted by another fiber or the like. A mixing ratio between the artificial protein fiber and the hydrophobic synthetic fiber is arbitrary, but the artificial protein fiber only needs to be mixed in the mixed region in an amount of 10 to 90% by mass. When the mixing ratio of the artificial protein fiber is less than 10% by mass, that is, when the mixing ratio of the hydrophobic synthetic fiber is more than 90% by mass, or when the mixing ratio of the artificial protein fiber is more than 90% by mass, that is, when the mixing ratio of the hydrophobic synthetic fiber is less than 10% by mass, the artificial protein fiber and the hydrophobic synthetic fiber may be insufficiently mixed, and it may be difficult to obtain desired spot absorbability. From this viewpoint, the mixing ratio between the artificial protein fiber and the hydrophobic synthetic fiber is generally artificial protein fiber : hydrophobic synthetic fiber = 10 to 90% by mass : 90 to 10% by mass (100% by mass in total), preferably 30 to 70% by mass : 70 to 30% by mass (100% by mass in total), and more preferably 40 to 60% by mass : 60 to 40% by mass (100% by mass in total). When the amount of the artificial protein fiber is too small, ability to absorb liquid tends to decrease, and when the amount of the artificial protein fiber is too large, spot absorbability tends to decrease.

[0034] The nonwoven fabric according to the present embodiment is produced by mixing an artificial protein fiber containing an artificial fibroin and a hydrophobic synthetic fiber. A method for producing a nonwoven fabric by mixing an artificial protein fiber and a hydrophobic synthetic fiber is not particularly limited, and any known method can be adopted. Specifically, the nonwoven fabric according to the present embodiment can be produced by a conventionally known production method such as a spunlace method, a needle punch method, or a thermal bond method. A typical production method is the following spunlace method. First, an artificial protein fiber and a hydrophobic synthetic fiber are uniformly mixed and caused to pass through a carding machine to obtain a fibrous web. This fibrous web is subjected to a high-pressure water flow to entangle the artificial protein fiber and the hydrophobic synthetic fiber with each other, and then dried to obtain the nonwoven fabric according to the present invention. When such a spunlace method is adopted, a nonwoven fabric in which the artificial protein fiber and the hydrophobic synthetic fiber are firmly entangled with each other and which has high tensile strength without using an adhesive or the like can be obtained.

[0035] The nonwoven fabric according to the present embodiment can be used for various applications. Specifically, the nonwoven fabric is used as a scratch pad, a surface material of a sanitary material such as a sanitary napkin or a disposable diaper, a wiping cloth, or the like.

Advantageous Effects of Invention

[0036] The nonwoven fabric according to the present invention has an effect of excellent spot absorbability and high water absorption ability. In addition, the nonwoven fabric according to the present invention also has an effect of favorable skin contact because an artificial protein fiber is contained in the nonwoven fabric. According to the production method according to the present invention, a production process is simplified, whereby easy production can be performed, and improvement in productivity can be desired.

Examples

[0037] Hereinafter, the present invention will be described more specifically based on Examples and the like. Note that the present invention is not limited to the following Examples.

(1) Production of modified fibroin

(Synthesis of nucleic acid encoding modified fibroin and construction of expression vector)

[0038] A modified fibroin 1 having the amino acid sequence set forth in in SEQ ID NO: 40 (average hydropathy index: 0.466) and a modified fibroin 2 having the amino acid sequence set forth in in SEQ ID NO: 15 (average hydropathy index: -0.801) were designed.

[0039] Each of nucleic acids encoding the designed two types of modified fibroins was synthesized. In each of the nucleic acids, an NdeI site was added to a 5' terminal and an EcoRI site was added downstream of a stop codon. Each of the five types of nucleic acids was cloned with a cloning vector (pUC118). Thereafter, each of the nucleic acids was cleaved at NdeI and EcoRI by a restriction enzyme treatment and then recombined with a protein expression vector pET-22b(+) to obtain an expression vector.

(Expression of modified fibroin)

[0040] Escherichia coli BLR (DE3) was transformed with each of the obtained expression vectors. The transformed Escherichia coli was cultured for 15 hours in a 2 mL LB culture medium containing ampicillin. The culture solution was added to a 100 mL seed culture medium containing ampicillin (Table 2) such that OD600 reached 0.005. The culture solution was maintained at a temperature of 30°C and subjected to flask culture (for about 15 hours) until OD600 reached 5, thereby obtaining a seed culture solution.

[Table 2]

Seed culture medium	
Reagent	Concentration (g/L)
Glucose	5.0
KH_2PO_4	4.0
K_2HPO_4	9.3
Yeast Extract	6.0
Ampicillin	0.1

The seed culture solution was added to ajar fermenter to which a 500 mL growing medium (Table 3 below) had been added such that OD600 reached 0.05. The culture solution was maintained at a temperature of 37°C and cultured while being controlled so as to have a pH of 6.9 constantly. In addition, a dissolved oxygen concentration in the culture solution was maintained at 20% of a saturated dissolved oxygen concentration.

[0041]

[Table 3]

Growing medium	
Reagent	Concentration (g/L)
Glucose	12.0
KH_2PO_4	9.0
$MgSO_4 \cdot 7H_2O$	2.4
Yeast Extract	15
$FeSO_4 \cdot 7H_2O$	0.04
$MnSO_4 \cdot 5H_2O$	0.04
$CaCl_2 \cdot 2H_2O$	0.04
ADEKANOL (ADEKA, LG-295S)	0.1 (mL/L)

[0042] Immediately after glucose in the growing medium was completely consumed, a feed solution (455 g/1 L of glucose, 120 g/1 L of Yeast Extract) was added thereto at a speed of 1 mL/min. The culture solution was maintained at a temperature of 37°C and cultured while being controlled so as to have a pH of 6.9 constantly. Culture was performed for 20 hours while the dissolved oxygen concentration in the culture solution was maintained at 20% of the saturated

dissolved oxygen concentration. Thereafter, 1 M isopropyl-β-thiogalactopyranoside (IPTG) was added to the culture solution such that a final concentration was 1 mM to induce expression of a target modified fibroin. When 20 hours had elapsed after the addition of IPTG, the culture solution was centrifuged, and bacterial cells were collected. SDS-PAGE was performed using bacterial cells prepared from the culture solutions before the addition of IPTG and after the addition of IPTG. Expression of the target modified fibroin which depended on the addition of IPTG was confirmed by appearance of a band having a size corresponding to the target modified fibroin.

(Purification of modified fibroin)

[0043] The bacterial cells collected two hours after the addition of IPTG were washed with a 20 mM Tris-HCl buffer (pH 7.4). The washed bacterial cells were suspended in a 20 mM Tris-HCl buffer (pH 7.4) containing about 1 mM PMSF, and the cells were disrupted with a high-pressure homogenizer (GEA Niro Soavi). The disrupted cells were centrifuged to obtain a precipitate. The obtained precipitate was washed with a 20 mM Tris-HCl buffer (pH 7.4) until the precipitate obtained a high purity. The washed precipitate was suspended in an 8 M guanidine buffer (8 M guanidine hydrochloride, 10 mM sodium dihydrogen phosphate, 20 mM NaCl, 1 mM Tris-HCl, pH 7.0) such that the precipitate had a concentration of 100 mg/mL, and dissolved therein by being stirred with a stirrer at 60°C for 30 minutes. After the precipitate was dissolved, the resulting solution was dialyzed with water using a dialysis tube (cellulose tube 36/32 manufactured by Sanko Junyaku Co., Ltd.). A white aggregated protein obtained after dialysis was collected by centrifugation. Water was removed from the collected aggregated protein by a freeze-dryer to obtain freeze-dried powders of two types of modified fibroins 1 and 2 having different amino acid sequences.

(2) Production of artificial protein fiber

(Preparation of dope solution)

[0044] Dimethyl sulfoxide (DMSO) in which LiCl was dissolved so as to have a concentration of 4.0% by mass was prepared as a solvent, and the freeze-dried powders of the modified fibroins 1 and 2 were added thereto so as to have a concentration of 18% by mass or 24% by mass, and dissolved therein for three hours using a shaker. Thereafter, insoluble matters and foams were removed to obtain two types of modified fibroin solutions.

(Spinning)

[0045] The obtained two types of modified fibroin solutions were used as dope solutions (spinning dopes), and dry-wet spinning and drawing were performed using a known dry-wet apparatus to produce two types of artificial protein fibers containing the two types of modified fibroins, respectively. Then, these artificial protein fibers 1 and 2 were each wound around a bobbin. Out of these two types of artificial protein fibers, an artificial protein fiber containing the modified fibroin 1 is referred to as an artificial protein fiber 1, and an artificial protein fiber containing the modified fibroin 2 is referred to as an artificial protein fiber 2. Conditions of dry-wet spinning are as described below.

Temperature of coagulation liquid (methanol): 5 to 10°C
Draw ratio: 4.52 times
Drying temperature: 80°C

[0046] Next, the artificial protein fibers 1 and 2 obtained as described above and wound around the bobbins were pulled out from the bobbins, respectively. A plurality of the artificial protein fibers 1 was bundled, and a plurality of the artificial protein fibers 2 was bundled. The obtained bundles were each cut into a length of 50 mm with a tabletop fiber cutter to prepare a large number of artificial protein short fibers 1 and a large number of artificial protein short fibers 2. Thereafter, these two types of artificial protein 1 and artificial protein short fibers 2 were each immersed in water at 40°C for one minute to be crimped, and then dried at 40°C for 18 hours to obtain a large number of crimped artificial protein short fibers 1 and a large number of crimped artificial protein fibers 2. These two types of artificial protein short fibers 1 and 2 each had a fineness of about 1.4 to 1.8 dtex.

Example 1

[0047] As a hydrophobic synthetic fiber, a polyester fiber ("Tetron T471" manufactured by Toray Industries, Inc.) having a fineness of 1.6 dtex and a fiber length of 51 mm was prepared. This polyester fiber has an official moisture regain of about 0.4%. Subsequently, 50% by mass of the crimped artificial protein short fibers 1 containing the modified fibroin 1 having the amino acid sequence of SEQ ID NO: 40, obtained as described above, and 50% by mass of the polyester

fibers were uniformly mixed and then caused to pass through a carding machine to obtain a fibrous web. While this fibrous web was placed on a conveyor and conveyed, a water flow was applied to the fibrous web at a water pressure of 2 MPa. Thereafter, the fibrous web was reversed, and a water flow was applied to the fibrous web at a water pressure of 4 MPa to preliminarily entangle the fibers with each other. Thereafter, the fibrous web was further reversed, and a water flow was applied to the fibrous web at a water pressure of 6 MPa to entangle the fibers with each other. Thereafter, the fibrous web was dried to obtain a nonwoven fabric having a basis weight of 62 g/m^2.

Example 2

[0048] A nonwoven fabric having a basis weight of 72 g/m^2 was obtained in a similar manner to Example 1 except that a polypropylene fiber ("Polypro PN" manufactured by Daiwabo Holdings Co., Ltd.) having a fineness of 1.7 dtex and a fiber length of 44 mm was used instead of the polyester fiber. Note that the polypropylene fiber has an official moisture regain of about 0.0%.

Example 3

[0049] A nonwoven fabric having a basis weight of 69 g/m^2 was obtained in a similar manner to Example 1 except that a polylactic acid fiber ("Terramac PL01" manufactured by Unitika Corporation) having a fineness of 1.7 dtex and a fiber length of 51 mm was used instead of the polyester fiber. Note that the polylactic acid fiber has an official moisture regain of about 0.5%.

Example 4

[0050] A nonwoven fabric having a basis weight of 55 g/m^2 was obtained in a similar manner to Example 1 except that the crimped artificial protein short fiber 2 containing the modified fibroin 2 having the amino acid sequence of SEQ ID NO: 15 was used instead of the crimped artificial protein short fiber 1.

Example 5

[0051] A nonwoven fabric having a basis weight of 55 g/m^2 was obtained in a similar manner to Example 4 except that a polylactic acid fiber ("Terramac PL01" manufactured by Unitika Corporation) having a fineness of 1.7 dtex and a fiber length of 51 mm was used instead of the polyester fiber.

Comparative Example 1

[0052] A nonwoven fabric having a basis weight of 64 g/m^2 was obtained in a similar manner to Example 1 except that a fibrous web formed only of the crimped artificial protein short fiber 1 was used without using the polyester fiber.

Comparative Example 2

[0053] A nonwoven fabric having a basis weight of 57 g/m^2 was obtained in a similar manner to Example 1 except that a polyacrylonitrile fiber ("Vonnel H129" manufactured by Mitsubishi Chemical Corporation), which is a hydrophilic synthetic fiber, having a fineness of 1.0 dtex and a fiber length of 44 mm was used instead of the polyester fiber. Note that the polyacrylonitrile fiber has an official moisture regain of about 2.0%.

Comparative Example 3

[0054] A nonwoven fabric having a basis weight of 64 g/m^2 was obtained in a similar manner to Example 1 except that a rayon fiber ("HOPE NWD" manufactured by Omikenshi Co., Ltd.), which is a hydrophilic synthetic fiber, having a fineness of 1.7 dtex and a fiber length of 40 mm instead of the artificial protein short fiber 1. Note that the rayon fiber has an official moisture regain of about 11.0%.

[0055] The nonwoven fabrics obtained in Example 1 to 5 and Comparative Example 1 to 3 were measured for the following physical properties, and results thereof are as presented in Table 4.

[Water absorption ability]

[0056] Water absorption ability (%) was measured in accordance with JIS L 1912.

[Suction height]

[0057] A suction height (mm) after one minute was measured for an MD direction (a direction in which the fibrous web is conveyed) of the nonwoven fabric and a CD direction (direction orthogonal to the MD direction) thereof in accordance with JIS L 1912.

[Table 4]

	Water absorption ability	Suction height	
		MD direction	CD direction
Example 1	1064	2	1
Example 2	603	6	1
Example 3	613	0	0
Example 4	491	0	0
Example 5	370	0	0
Comparative Example 1	505	47	54
Comparative Example 2	982	40	51
Comparative Example 3	1010	30	25

[0058] As can be seen from the results in Table 4, the nonwoven fabrics according to Examples have lower suction heights in both the MD direction and the CD direction than the nonwoven fabrics according to Comparative Examples. This means that water does not diffuse in a plane direction even when water is dropped onto the nonwoven fabrics according to Examples. Therefore, when each of the nonwoven fabrics according to Examples is used for a surface material of a sanitary material such as a sanitary napkin, it is possible to provide a sanitary material which absorbs and transmits a body fluid well, but does not cause the body fluid to diffuse in a plane direction, and does not easily adhere to the skin.

Claims

1. A nonwoven fabric having a region formed by mixing an artificial protein fiber containing an artificial fibroin and a hydrophobic synthetic fiber.

2. The nonwoven fabric according to claim 1, wherein the artificial protein fiber containing an artificial fibroin and the hydrophobic synthetic fiber are mixed in an entire region.

3. The nonwoven fabric according to claim 1 or 2, wherein the artificial protein fiber containing an artificial fibroin and the hydrophobic synthetic fiber are uniformly mixed.

4. The nonwoven fabric according to any one of claims 1 to 3, wherein the artificial fibroin is a modified spider silk fibroin.

5. The nonwoven fabric according to any one of claims 1 to 4, wherein the artificial fibroin has an average hydropathy index of -1.0 or more.

6. The nonwoven fabric according to claim 5, wherein the artificial fibroin has an average hydropathy index of more than 0.0.

7. The nonwoven fabric according to any one of claims 1 to 6, comprising the artificial protein fiber in an amount of 10 to 90% by mass.

8. The nonwoven fabric according to any one of claims 1 to 7, wherein the hydrophobic synthetic fiber has an official moisture regain of less than 2%.

9. The nonwoven fabric according to any one of claims 1 to 8, wherein the hydrophobic synthetic fiber is at least one fiber selected from the group consisting of a polyethylene terephthalate fiber, a polypropylene fiber, and a polylactic acid fiber.

10. A method for producing a nonwoven fabric, comprising mixing an artificial protein fiber containing an artificial fibroin and a hydrophobic synthetic fiber.

11. The method for producing a nonwoven fabric according to claim 10, wherein a water flow is applied to a fibrous web formed by mixing an artificial protein fiber containing an artificial fibroin and a hydrophobic synthetic fiber to entangle the artificial protein fiber and the hydrophobic synthetic fiber with each other, thereby further promoting mixing.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2021/020191

A. CLASSIFICATION OF SUBJECT MATTER
D04H 1/4266(2012.01)i; D04H 1/4291(2012.01)i; D04H 1/435(2012.01)i; D04H 1/492(2012.01)i
FI: D04H1/4266; D04H1/4291; D04H1/435; D04H1/492
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
D04H1/00-18/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan	1922-1996
Published unexamined utility model applications of Japan	1971-2021
Registered utility model specifications of Japan	1996-2021
Published registered utility model applications of Japan	1994-2021

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2019/194230 A1 (SPIBER INC.) 10 October 2019 (2019-10-10) claims 1, 7, 8, paragraph [0190]	1-11
A	WO 2020/067513 A1 (SPIBER INC.) 02 April 2020 (2020-04-02) claims 1, 9, paragraph [0219]	1-11
A	JP 2010-222726 A (EMU EMU EEMU KK) 07 October 2010 (2010-10-07) claim 1, example 1	1-11
A	JP 9-188972 A (JIYOUMOU NENSHI KK) 22 July 1997 (1997-07-22)	1-11
A	JP 58-132154 A (UNI-CHARM CORP.) 06 August 1983 (1983-08-06)	1-11
A	CN 101831762 A (SUZHOU UNIVERSITY) 15 September 2010 (2010-09-15)	1-11

☐ Further documents are listed in the continuation of Box C.
☒ See patent family annex.

* Special categories of cited documents:
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"&" document member of the same patent family

Date of the actual completion of the international search 08 September 2021 (08.09.2021)	Date of mailing of the international search report 21 September 2021 (21.09.2021)
Name and mailing address of the ISA/ Japan Patent Office 3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan	Authorized officer Telephone No.

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/JP2021/020191

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
WO 2019/194230 A1	10 Oct. 2019	(Family: none)	
WO 2020/067513 A1	02 Apr. 2020	(Family: none)	
JP 2010-222726 A	07 Oct. 2010	(Family: none)	
JP 9-188972 A	22 Jul. 1997	(Family: none)	
JP 58-132154 A	06 Aug. 1983	GB 2114174 A DE 3302709 A FR 2520765 A IT 8367089 DO	
CN 101831762 A	15 Sep. 2010	(Family: none)	

Form PCT/ISA/210 (patent family annex) (January 2015)

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- JP 2008125602 A **[0003]**
- WO 2020067546 A **[0013] [0030]**

Non-patent literature cited in the description

- **KYTE J ; DOOLITTLE R.** A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.,* 1982, vol. 157, 105-132 **[0023]**