

【DESCRIPTION】**【Invention Title】**

CELL SCAFFOLD FOR PRODUCING CULTIVATED MEAT AND
PRODUCTION METHOD THEREFOR

5 **【Technical Field】**

[1] The present invention relates to a cell scaffold for producing cultivated meat, a method for producing the same, and a method for producing cultivated meat using the same.

【Background Art】

10 [2] Meat is an important source of water and nutrients such as proteins, lipids, iron, zinc, and vitamin B12, regardless of the type of animal. In a situation where plant-based sources other than soybeans are limited, humans have historically utilized meat as an important food source to supplement
15 essential amino acids. Beyond its nutritional importance, meat has also played a significant role in the cultural development of diverse societies and cuisines. Meat is composed of about 65 to 80% water, 16 to 22% protein, and 1.5 to 13% fat, and the degree of fat distributed within muscle
20 (intramuscular fat, marbling) is an important factor that determines meat quality and flavor.

[3] Despite these nutritional advantages of meat, there has been increasing concern regarding livestock rearing with high greenhouse gas emissions due to global trends such as carbon
25 neutrality and animal welfare. According to the Food and

Agriculture Organization of the United Nations (FAO), the livestock industry not only uses a substantial portion of global agricultural land but also accounts for about 14.5% of global greenhouse gas (GHG) emissions. In addition, wastewater and the like discharged during livestock rearing have a significant impact on water and soil pollution.

[4] As a solution to these issues, artificial meat produced by culturing stem cells derived from animals in a laboratory has attracted increasing attention. Cultivated meat (artificial meat), which is also referred to as farmed meat or "green meat", not only contributes to improvements in animal welfare but also may significantly reduce greenhouse gas emissions associated with meat production. According to some estimates, greenhouse gas emissions may be reduced by up to 96% as large-scale deforestation and the like for livestock rearing are reduced.

[5] In order to produce cultivated meat that is close to actual meat, it is necessary to realize a similar texture; however, technologies capable of mimicking a complex marbling structure and economically producing large-sized meat on a large scale are still insufficient. The primary reason for this is that the materials currently used as cell scaffolds for cultivated meat are expensive, and there is a lack of technologies capable of realistically mimicking various components of meat, such as muscle, fat, and blood vessels.

【Disclosure】**【Technical Problem】**

[6] An object of the present invention is to provide a cell scaffold for producing cultivated meat. Specifically, the present invention provides a cell scaffold in which a polymer capable of forming reversible physicochemical bonds through a self-healing process is introduced, adipocytes and myocytes are cultured separately, and the cultured cells are assembled with each other through the self-healing process, thereby enabling the formation of marbling similar to that of actual meat. In addition, the present invention provides a cell scaffold for producing cultivated meat that allows free adjustment of the sizes and contents of fat portions and muscle portions of cultivated meat and is capable of forming marbling close to that of actual meat, by enabling assembly through a self-healing process, a method for producing the same, and a method for producing cultivated meat using the same.

[7] In addition, the present invention provides a self-healing polymer in which specific chemical or physical properties are introduced to enable a self-healing function while allowing cell culture. As an example, by using chemical bonds capable of cleavage and re-formation, a material may undergo self-healing in a manner similar to a healing process in biological tissue. This exhibits characteristics close to biological properties and shows substantial similarity to the

regenerative capacity of biological tissue. Accordingly, it is intended to produce cultivated meat having a structure similar to that of actual meat by using such a self-healing polymer as a cell scaffold for forming a cultivated meat structure.

[8] Another object of the present invention is to provide a biocompatible self-healing hydrogel. As an example, the present invention provides a biocompatible self-healing hydrogel that is capable of inducing cell adhesion or gel degradation and is usable as a scaffold for inducing tissue regeneration.

【Technical Solution】

[9] In one general aspect, a cell scaffold for producing cultivated meat includes a self-healing hydrogel, wherein cross-sections of at least two self-healing hydrogels that are identical to or different from each other are capable of being assembled with each other by forming reversible physicochemical bonds through a self-healing process.

[10] In one embodiment, the cell scaffold for producing cultivated meat may include a first self-healing hydrogel in which myocytes are cultured and a second self-healing hydrogel in which adipocytes are cultured.

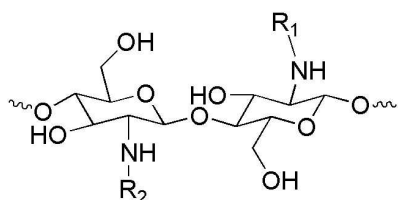
[11] In one embodiment, the self-healing hydrogel may form reversible physicochemical bonds through a self-healing process in one or more environments selected from temperature

and pH ranges in which cells are culturable.

[12] In one embodiment, the self-healing hydrogel may include a unit (A) derived from chitosan substituted with a boronic acid compound, and a unit (B) derived from a polymer containing a hydroxy group.

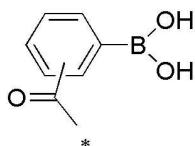
[13] In one embodiment, the unit (A) derived from chitosan substituted with a boronic acid compound may include a compound represented by the following Chemical Formula 1.

[14] [Chemical Formula 1]



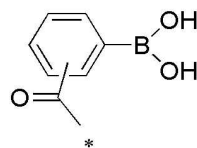
[15]

[16] In Chemical Formula 1, R_1 and R_2 are each independently



selected from hydrogen or

, and



[17] at least one of R_1 and R_2 is .

[18] In one embodiment, the unit (A) derived from chitosan substituted with a boronic acid compound may have a substitution rate of the boronic acid compound according to the following Equation 1 of 10 to 50%.

[19] [Equation 1]

[20] Substitution rate = (Number of moles of amines substituted with boronic acid compound/Total number of moles

of amines in chitosan) $\times 100$

[21] In one embodiment, the self-healing hydrogel may have a gel fraction of 50 to 90%, but is not limited thereto.

[22] In one embodiment, the cell scaffold may further include
5 a strength-reinforcing material.

[23] In one embodiment, the cell scaffold may be capable of culturing one or two or more cells selected from the group consisting of myoblasts, adipocytes, fibroblasts, myocytes, mesenchymal stem cells (MSCs), induced pluripotent stem cells
10 (iPSCs), satellite cells, endothelial cells, and embryonic stem cells or the like.

[24] In one embodiment, a first component of the self-healing hydrogel in which myocytes are cultured (proliferated) may have a Young's modulus of 200 to 400 kPa, and a second component of
15 the self-healing hydrogel in which adipocytes are cultured (proliferated) may have a Young's modulus of 50 to 180 kPa, but these components are not limited thereto.

[25] In another general aspect, there is provided cultivated meat cultured using the cell scaffold for producing cultivated
20 meat according to one embodiment.

[26] In one embodiment, the cultivated meat may have marbling similar to that of actual meat.

[27] In still another general aspect, a method for producing a cell scaffold using a self-healing hydrogel includes mixing
25 a chitosan resin precursor substituted with a boronic acid

compound and a polymer containing a hydroxy group, and then inducing crosslinking by adjusting a pH.

[28] In one embodiment, the method may further include, after the mixing, freezing and then thawing the mixture.

5 [29] In one embodiment, during the mixing, a strength-reinforcing material may be further added.

[30] In still another general aspect, a method for producing cultivated meat using a cell scaffold including a self-healing hydrogel includes: preparing a first self-healing hydrogel and
10 a second self-healing hydrogel, respectively, having different mixing ratios of a chitosan resin precursor substituted with a boronic acid compound and a polymer containing a hydroxy group; and

[31] culturing myocytes in one of the first self-healing
15 hydrogel and the second self-healing hydrogel, and culturing and differentiating adipocytes in the other one.

[32]

[33] In one embodiment, in the preparing of the first self-healing hydrogel and the second self-healing hydrogel, a
20 strength-reinforcing material may be further added.

[34] In one embodiment, the method may further include, in the preparing of the first self-healing hydrogel and the second self-healing hydrogel, freezing and then thawing the first self-healing hydrogel and the second self-healing hydrogel.

25 [35] In one embodiment, the first self-healing hydrogel may

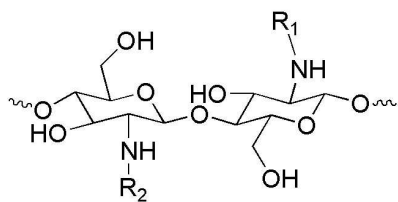
have a Young's modulus of 200 to 400 kPa, and the second self-healing hydrogel may have a Young's modulus of 50 to 180 kPa, but these self-healing hydrogels are not limited thereto.

[36] In one embodiment, the first self-healing hydrogel and
5 the second self-healing hydrogel may be assembled with each other through a self-healing process by culturing myocytes in one of the first self-healing hydrogel and the second self-healing hydrogel, culturing and differentiating adipocytes in the other one, and then stacking the self-healing
10 hydrogels or arranging the self-healing hydrogels to be in contact with each other.

[37] In one embodiment, in a state in which the first self-healing hydrogel and the second self-healing hydrogel are stacked or arranged to be in contact with each other so as to
15 be assembled with each other through a self-healing process, myocytes may be cultured in one of the first self-healing hydrogel and the second self-healing hydrogel, and adipocytes may be cultured and differentiated in the other one.

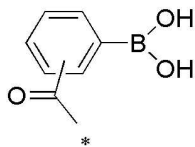
[38] In still another general aspect, a biocompatible
20 self-healing polymer includes a unit (A) derived from chitosan to which a boronic acid compound including a compound represented by the following Chemical Formula 1 is bonded, and a unit (B) derived from a polymer containing a hydroxy group.

[39] [Chemical Formula 1]

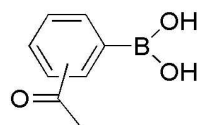


[40]

[41] In Chemical Formula 1, R_1 and R_2 are each independently



selected from hydrogen or



, and

[42] at least one of R_1 and R_2 is

5 [43] In one embodiment, the self-healing polymers may be assembled with each other at a pH of 6.5 to 12.

[44] In one embodiment, the unit (A) derived from chitosan substituted with a boronic acid compound may have a substitution rate of the boronic acid compound according to the following

10 Equation 1 of 10 to 50%, but is not limited thereto.

[45] [Equation 1]

[46] Substitution rate = (Number of moles of amines substituted with boronic acid compound/Total number of moles of amines in chitosan) $\times$ 100

15 [47] In one embodiment, the self-healing polymer may further include a strength-reinforcing material.

[48] In one embodiment, the self-healing polymer may have a Young's modulus of 50 to 400 kPa, but is not limited thereto.

【Advantageous Effects】

20 [49] The biocompatible self-healing hydrogel according to one

embodiment of the present invention has biocompatibility, is capable of cell culture, and is capable of self-healing under conditions in which cell culture is possible. Accordingly, scaffolds suitable for fat and muscle, respectively, may be provided, and cultivated meat in which marbling may be formed like actual meat may be produced by allowing a portion in which fat is cultured and a portion in which muscle is cultured to be assembled with each other through a self-healing process under cell culture conditions. In addition, the size may be increased, through a self-healing process, by allowing portions in which fat is cultured to be assembled with each other, or by allowing portions in which muscle is cultured to be assembled with each other.

[50] In addition, a biocompatible self-healing hydrogel according to one embodiment of the present invention and a cell scaffold for producing cultivated meat using the same may join together cut surfaces without applying a separate chemical method such as an adhesive or additional energy. Accordingly, the self-healing hydrogel allows myocytes or adipocytes to be grown during cell culture solely by diffusion of substances, and then allows large-sized cultivated meat to be produced by joining portions including the grown myocytes or adipocytes at a desired size or position, and allows the content of fat in muscle and marbling to be freely adjusted.

25 **【Description of Drawings】**

[51] FIG. 1 is an infrared spectrum graph of chitosan ChsB(OH)₂ in which chitosan and 3-carboxybenzeneboronic acid are bonded according to Preparation Example 1.

[52] FIG. 2 is a graph showing ultraviolet-visible spectra of
5 chitosan ChsB(OH)₂ bonded to a boronic acid in a standard experiment and a comparative experiment according to Preparation Example 1.

[53] FIG. 3 is a graph showing absorbance intensities at 285 nm for different concentrations of 3-carboxybenzeneboronic
10 acid according to Preparation Example 1 to verify the substitution rate.

[54] FIG. 4 is a photograph showing self-healing and multi-module responsiveness of an N1 hydrogel according to an Example. FIG. 4a shows that three pieces including colored
15 pieces and an uncolored piece are fused into a single piece to form a large piece that may be lifted without separating. The dyes used are Brilliant Blue FCF and Amaranth Red 2. FIG. 4b shows that a single large hydrogel piece is reformed into three small pieces in heart, star, and flower shapes by cutting and
20 shaping alone. FIG. 4c shows liquefaction and gelation of the hydrogel in response to a pH change. FIGS. 4d and 4e show the responses of the hydrogel to glucose solutions at different concentrations. DMEM is an abbreviation for Dulbecco's modified Eagle's medium.

25 [55] FIGS. 5 to 7 show cell viability and proliferation in a

ChsB(OH)₂/PVA hydrogel according to Examples. FIG. 5 shows a cytotoxicity (biocompatibility) test of hydrogel components in two cell lines using Cell Counting Kit-8 (CCK-8). Low-density polyethylene (0.2 g mL⁻¹) and zinc diethyldithiocarbamate were used as a negative control and a positive control, respectively.

FIG. 6 shows results obtained by monitoring growth of a 3T3-L1 cell line on different hydrogel substrates for one week through absorbance from formation of orange formazan using CCK-8. FIG. 7 shows results obtained by monitoring growth of a C2C12 cell line on different hydrogel substrates for one week through absorbance from formation of orange formazan using CCK-8.

[56] FIG. 8 shows a comparison of the viability of 3T3-L1 cultured in F0.5 and the viability of C2C12 on an N1 ChsB(OH)₂/PVA hydrogel scaffold with Matrigel, which is a positive control, and shows the viability visualized by LIVE/DEAD staining using an optical microscope, according to an Example. Green (calcein AM) indicated by ① represents live cells, and blue (Hoechst 33342) indicated by ② represents cell nuclei.

[57] FIG. 9 shows differentiation of 3T3-L1 into adipose-like tissue on an F0.5 hydrogel and Matrigel, and differentiation of C2C12 into muscle-like tissue on an N1 hydrogel and Matrigel, according to an Example. The cells were observed on day 9 of differentiation using a fluorescence microscope. Red (Nile Red) indicated by ① represents lipid droplets, blue (Hoechst

33342) indicated by ⊙ represents cell nuclei, and green (Alexa Fluor 488) indicated by ⊙ represents myosin and F-actin.

[58] FIG. 10 is a photograph showing artificial meat produced using a self-healing hydrogel according to an Example. FIG.

5 10a shows individual N1 muscle-type cultures and F0.5 fat-type cultures differentiated for 28 days, and their appearance after frying. FIG. 10b shows an artificial meat model in which muscle-type tissue and fat-type tissue are alternately stacked to form two bilayers. Dimensions: x 1 cm, y 0.5 cm, z 0.35 cm.

10 The muscle tissue stained with carmine is distinguished from the adipose tissue.

【Description of Drawings】

[59] Hereinafter, the present invention will be described in detail. However, the following specific embodiments or
15 examples are only references to explain the present invention in detail, and the present invention is not limited thereto and may be implemented in various forms.

[60] In addition, unless otherwise defined, all the technical terms and scientific terms used have the same meanings as
20 commonly understood by those skilled in the art to which the present invention pertains. The terms used in the description of the present invention are merely used to effectively describe a specific embodiment, but are not intended to limit the present invention.

25 [61] In addition, unless the context clearly indicates

otherwise, singular forms used in the specification and the appended claims may be intended to include plural forms.

[62] In addition, unless explicitly described to the contrary, "comprising" any components will be understood to imply further
5 inclusion of other components rather than the exclusion of any other components.

[63] In addition, unless otherwise defined in the present disclosure, when a layer or a member is described as being "on" another layer or member, this includes not only a case where
10 the layer or member is in direct contact with the other layer or member, but also a case where another layer or another member is present between the two layers or the two members.

[64] In addition, as used in the present disclosure, the terms "about", "substantially", and the like are used to indicate that
15 manufacturing and material tolerances are at the stated numerical value or near the stated numerical value, and are also used to prevent an unscrupulous infringer from unjustly exploiting disclosures in which exact or absolute numerical values are presented to facilitate understanding of the present
20 invention.

[65] In addition, as used in the present disclosure, the term "self-healing" refers to adhesion achieved by a polymer forming reversible physicochemical bonds by itself under specific temperature or pH conditions, rather than by external factors
25 such as a separate adhesive.

[66] In addition, as used in the present disclosure, the term "hydrogel" is also referred to as a hydrated gel, and refers to a material that has a network structure in which polymers form three-dimensional crosslinking through physical bonds, such as hydrogen bonding, van der Waals forces, and hydrophobic interactions, or chemical bonds, such as covalent bonds, does not dissolve in an aqueous environment, and is capable of containing a substantial amount of water.

[67] Hereinafter, each configuration of the present invention will be described in more detail.

[68] [Cell Scaffold]

[69] An aspect of the present invention provides a cell scaffold for producing cultivated meat, the cell scaffold including a self-healing hydrogel, wherein cross-sections of at least two self-healing hydrogels that are identical to or different from each other are capable of being assembled with each other by forming reversible physicochemical bonds through a self-healing process.

[70] In one embodiment, the self-healing hydrogel may be a self-healing hydrogel that is capable of self-healing in temperature and pH ranges in which cells are culturable. In addition, the self-healing hydrogel may allow assembly without a separate adhesive by reversible physicochemical bonds of the hydrogel itself. In addition, the self-healing hydrogel is capable of culturing muscle tissue and adipose tissue, and

allows a cell scaffold in which muscle tissue is cultured and a cell scaffold in which adipose tissue is cultured to be assembled with each other through a self-healing process without the aid of an adhesive.

5 [71] In one embodiment, the cell scaffold for producing cultivated meat may include a first self-healing hydrogel in which myocytes are cultured and a second self-healing hydrogel in which adipocytes are cultured.

[72] In one embodiment, functional groups that form reversible
10 physicochemical bonds through the self-healing process may include one or two or more selected from the group consisting of hydrogen bonding, ionic bonding, boronic acid ester bonding, disulfide bonding, host-guest bonding, imine bonding, and acylhydrazone bonding, but are not limited thereto.

15 [73] In one embodiment, the self-healing hydrogels may be assembled with each other within a temperature range of 20°C to 40°C or 25°C to 28°C in which cells are culturable, and may form reversible physicochemical bonds capable of self-healing.

[74] In one embodiment, the self-healing hydrogels may be
20 assembled with each other within a pH range of 6.5 to 12 or 6.5 to 8 in which cells are culturable, and may form reversible physicochemical bonds capable of self-healing.

[75] The temperature and pH ranges are ranges in which cells are culturable, and the cell scaffold may be suitable for use
25 within the ranges, but these ranges are not limited thereto.

[76] The cell scaffolds according to one embodiment may be joined with each other through a self-healing process within temperature and pH ranges in which cells are culturable, and thus may be assembled with each other through the self-healing process without the need for a separate adhesive or the like. In addition, cross-sections of at least two cell scaffolds, which are identical to or different from each other, among a cell scaffold in which muscle tissue is cultured and a cell scaffold in which adipose tissue is cultured, may be stacked or positioned to be in contact with each other so as to be assembled with each other through a self-healing process.

[77] Accordingly, the size and content of a cell scaffold in which muscle tissue is cultured and a cell scaffold in which adipose tissue is cultured may be freely adjusted as desired. In addition, it is also possible to increase the size by assembling at least two cell scaffolds in which muscle tissue is cultured with each other. In addition, it is also possible to increase the size by assembling at least two cell scaffolds in which adipose tissue is cultured with each other.

[78] In one embodiment, the cell scaffold may be capable of culturing one or two or more cells selected from the group consisting of myoblasts, adipocytes, fibroblasts, myocytes, mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), satellite cells, endothelial cells, and embryonic stem cells, but is not limited thereto.

[79] The cell scaffold for producing cultivated meat may include a first self-healing hydrogel in which myocytes are cultured and a second self-healing hydrogel in which adipocytes are cultured.

5 [80] In one embodiment, myocytes may be cultured in one of the first self-healing hydrogel and the second self-healing hydrogel, adipocytes may be cultured in the other one, and thereafter, these self-healing hydrogels may be joined to each other so as to form marbling similar to that of actual meat.

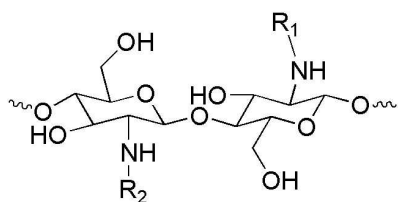
10 [81] In one embodiment, the self-healing hydrogel may form marbling similar to that of actual meat by joining, in an irregular pattern, a first component of the self-healing hydrogel in which myocytes are cultured (proliferated) and a second component of the self-healing hydrogel in which
15 adipocytes are cultured (proliferated). The self-healing hydrogel may allow the first component of the self-healing hydrogel and the second component of the self-healing hydrogel to be assembled with each other within a temperature range in which cells are cultured, and accordingly may form marbling
20 similar to that of actual meat.

[82] In one embodiment, the self-healing hydrogel may include a unit (A) derived from chitosan substituted with a boronic acid compound, and a unit (B) derived from a polymer containing a hydroxy group.

25 [83] In one embodiment, the unit (A) derived from chitosan

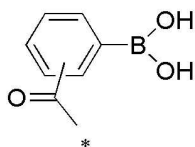
substituted with a boronic acid compound may include a compound represented by the following Chemical Formula 1, but is not limited thereto.

[84] [Chemical Formula 1]



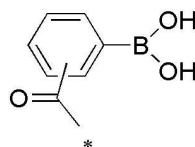
5 [85]

[86] In Chemical Formula 1, R_1 and R_2 are each independently



selected from hydrogen or

, and

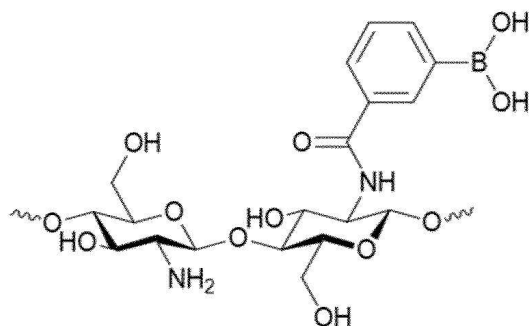


[87] at least one of R_1 and R_2 is .

[88] In one embodiment, the unit (A) represented by Chemical

10 Formula 1 may be represented by the following Chemical Formula 2, but is not limited thereto.

[89] [Chemical Formula 2]



[90]

[91] In one embodiment, the unit (A) derived from chitosan

15 substituted with a boronic acid compound may have a substitution

rate of the boronic acid compound according to the following Equation 1 of 10% or more, 15% or more, 20% or more, 25% or more, 30% or more, 35% or more, 50% or less, 45% or less, or 40% or less, and may be any value between the above values. For example, the substitution rate of the boronic acid compound may be 10 to 50%, 15 to 45%, or 20 to 30%, but is not limited thereto. Within the substitution rate satisfying the above range, unsubstituted amines of chitosan may be stabilized so as to maintain boronic acid ester bonds of the boronic acid compound.

Accordingly, self-healing may be achieved.

[92] [Equation 1]

[93]
$$\text{Substitution rate} = (\text{Number of moles of amines substituted with boronic acid compound} / \text{Total number of moles of amines in chitosan}) \times 100$$

[94] In Equation 1, the total number of moles of amines of chitosan refers to the number of moles of amines present without being acetylated when chitosan is deacetylated. The total number of moles of amines of chitosan may be calculated from the content of glucosamine.

[95] In one embodiment, the self-healing hydrogel may include the unit (A) derived from chitosan substituted with a boronic acid compound and the unit (B) derived from a polymer containing a hydroxy group in a weight ratio of 0.1 to 0.9:1, 0.15 to 0.9:1, 0.3 to 0.9:1, or 0.6 to 0.9:1, but is not limited thereto.

[96] In one embodiment, the self-healing hydrogel may have a

dual network formed through boronic acid ester bonds and hydrogen bonds.

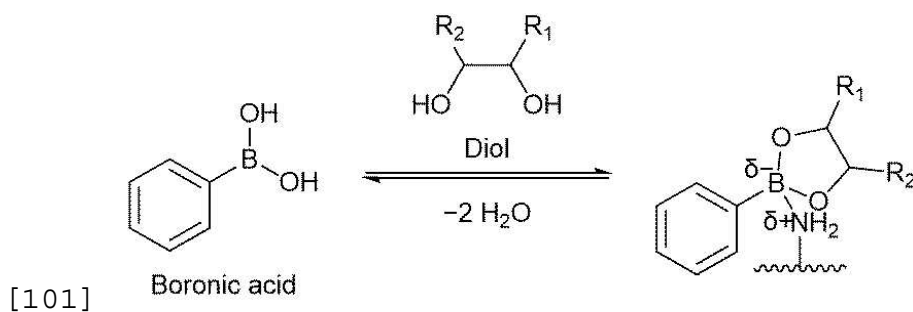
[97] That is, a dual network may be formed through boronic acid ester bonds resulting from a reaction between the unit (A) and the unit (B), and hydrogen bonds between hydroxyl groups of the polymer containing a hydroxy group. When such a dual network is formed, self-healing may be possible due to boronic acid-diol ester bonds, which are flexible and dynamic covalent bonds. In addition, mechanical strength may be further improved through hydrogen bonds between hydroxyl groups of the polymer containing a hydroxy group.

[98] In Chemical Formula 1, the bonding between chitosan and the boronic acid compound may be stabilized through a Lewis acid-base interaction between an amino group of chitosan and boron of the boronic acid compound. Through this, boronic acid-diol ester bonds that were conventionally stabilized only under basic conditions (for example, pH 8.5 or higher) may form sufficiently stable boronic acid-diol ester bonds even at neutral pH (between 6 and 8) by coordination bonding with the chitosan.

[99] In the case of the bonding between the chitosan and the boronic acid compound, stabilization may be achieved by forming boronic acid-diol ester bonds through bonding with diol groups of a polyol polymer compound such as polyvinyl alcohol. Such boronic acid-diol ester bonds cannot be stabilized in the

absence of an amine group of chitosan and inevitably dissociate; however, the present invention enables formation of boronic acid-diol ester bonds that are very stable by the presence of chitosan.

5 [100] The following schematically illustrates stabilization of a boronic acid-diol ester complex by a nitrogen atom of an amine group of chitosan. Here, diol refers to a substance having a hydroxy group, and in the present invention, may refer to a polymer containing a hydroxy group, exemplified by polyvinyl
10 alcohol.



[102] In order to bind the chitosan and the boronic acid compound, a crosslinker may be used, and for example, a zero-length crosslinker may be used. Examples of the
15 crosslinker include, but are not limited to, *N,N'*-carbonyldiimidazole (CDI), *N,N'*-dicyclohexylmethanediimine (DCC), *N*-hydroxysuccinimide (NHS), *N*-hydroxysulfosuccinimide (sulfo-NHS), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC).

20 [103] The chitosan may have, for example, a diameter of 1 to 500 nm, 5 to 300 nm, or 10 to 100 nm, and a length of 1 μ m to

10 mm, 1 μ m to 5 mm, or 5 μ m to 5 mm, but is not limited thereto. In addition, the chitosan may have a molecular weight of 100 kDa or more, 150 kDa or more, 160 kDa or more, 170 kDa or more, 180 kDa or more, 190 kDa or more, 200 kDa or more, 250 kDa or
5 more, 300 kDa or more, 310 kDa or more, 500 kDa or less, 450 kDa or less, 400 kDa or less, 390 kDa or less, 380 kDa or less, 370 kDa or less, 360 kDa or less, or 350 kDa or less, and the molecular weight may be any value between the above values. For example, chitosan having a molecular weight of 100 to 500 kDa,
10 150 to 400 kDa, or 190 to 310 kDa may be used, but is not limited thereto.

[104] In addition, the chitosan may be deacetylated, and may have a degree of deacetylation of 70% or more, 80% or more, or 90% or less, and the degree of deacetylation may be any value
15 between the above values. For example, the degree of deacetylation may be 70 to 90% or 80 to 90%, but is not limited thereto.

[105] The boronic acid compound may preferably have a carboxylic acid group in order to allow formation of the boronic
20 acid-diol ester bond, and for example, a boronic acid having a substituent containing a carboxylic acid group may be used. Specific examples thereof include, but are not limited to, 2-carboxybenzeneboronic acid, 3-carboxybenzeneboronic acid, and 4-carboxybenzeneboronic acid.

25 [106] The polymer containing a hydroxy group is preferably a

polymer that contains a hydroxy group and has biocompatibility, and for example, polyvinyl alcohol may be used. The polyvinyl alcohol is not limited, but may have a weight average molecular weight of 10,000 g/mol or more and 100,000 g/mol or less, or
5 any value between the above values. For example, polyvinyl alcohol having a weight average molecular weight of 10,000 to 100,000 g/mol, 30,000 to 100,000 g/mol, 50,000 to 100,000 g/mol, 70,000 to 100,000 g/mol, 80,000 to 100,000 g/mol, or 89,000 to 98,000 g/mol may be used.

10 [107] In one embodiment, the cell scaffold may further include a strength-reinforcing material as needed. The strength-reinforcing material is used to improve the strength of the cell scaffold, and examples thereof include cellulose nanocrystals, cellulose nanofibers, chitosan nanowhiskers,
15 and nanochitin and the like. In addition, any strength-reinforcing material may be used without limitation as long as it is a strength-reinforcing material that is commonly usable in a cell scaffold. The content of the strength-reinforcing material is not limited, but for example,
20 may be 1 wt% or less, 0.9 wt% or less, 0.5 wt% or less, 0.3 wt% or less, 0.2 wt% or less, or 0.1 wt% or less, based on the total weight of the cell scaffold. For example, the strength-reinforcing material may be used in an amount of 0.01 to 1 wt%, 0.05 to 1 wt%, or 0.1 to 0.5 wt%, and the content of
25 the strength-reinforcing material may be any value between the

above values. The content of the strength-reinforcing material is not limited to the above ranges as long as it is an amount that may be used to adjust strength so as to have strength suitable for culturing and differentiating myocytes
5 or adipocytes.

[108] Without being limited thereto, for example, the first component of the self-healing hydrogel in which myocytes are cultured (proliferated) may have a Young's modulus of 200 to 400 kPa, and the second component of the self-healing hydrogel
10 in which adipocytes are cultured (proliferated) may have a Young's modulus of 50 to 180 kPa. The above ranges are preferable in that strength suitable for culturing and differentiating myocytes and adipocytes may be provided within the ranges, but are not limited thereto. The Young's modulus
15 refers to a value measured according to a method described below.

[109] In one embodiment, the self-healing hydrogel may have a gel fraction of 50% or more, 51% or more, 55% or more, 60% or more, 63% or more, 65% or more, 70% or more, 73% or more, 75%
20 or more, 77% or more, 80% or more, 83% or more, 84% or more, 90% or less, or any value between the above values, but is not limited thereto. For example, the gel fraction of the self-healing hydrogel may be 50 to 90%. The gel fraction refers to a value measured by a method described above.

25 [110] In one embodiment, the cell scaffold may be in the form

of a scaffold, a microbead, a hydrogel, or a fiber, but is not limited thereto.

[111] [Method for Producing Cell Scaffold]

[112] Another aspect of the present invention provides a method
5 for producing a cell scaffold using a self-healing hydrogel,
the method including mixing a chitosan resin precursor
substituted with a boronic acid compound and a polymer
containing a hydroxy group, and then inducing crosslinking by
adjusting a pH.

10 [113] In one embodiment, when mixing the chitosan resin
precursor substituted with a boronic acid compound and the
polymer containing a hydroxy group, the Young's modulus of the
cell scaffold may be adjusted to a desired range by adjusting
a mixing ratio thereof. In one embodiment, the content of the
15 chitosan resin precursor substituted with a boronic acid
compound relative to the polymer containing a hydroxy group may
be in a weight ratio of 0.1 to 0.9:1, but is not limited thereto.
For example, the weight ratio may be 0.15 to 0.9:1, 0.3 to 0.9:1,
or 0.6 to 0.9:1.

20 [114] In addition, the Young's modulus may be adjusted to a
desired range by further adding a strength-reinforcing material
as needed. The strength-reinforcing material is not limited
as long as it is a material that is commonly applicable to a
cell scaffold, and the content thereof may be, for example, 1
25 wt% or less or 0.5 wt% or less based on the total weight of the

cell scaffold; however, the content may be used in an amount for adjusting a desired strength, but is not limited thereto.

[115] In addition, the method may further include freezing and then thawing the prepared self-healing hydrogel as needed, thereby adjusting mechanical strength, for example, a Young's modulus, to a desired range. By including such a freezing-and-thawing process, hydrogen bonding between chains of polyvinyl alcohol may be induced, thereby increasing mechanical properties of the hydrogel.

[116] In one embodiment, a first component of the self-healing hydrogel in which myocytes are cultured (proliferated) may be adjusted to have a Young's modulus of 200 to 400 kPa, and a second component of the self-healing hydrogel in which adipocytes are cultured (proliferated) may be adjusted to have a Young's modulus of 50 to 180 kPa. The above ranges are preferable in that cell culture is facilitated and a texture similar to that of actual meat may be provided, but are not limited thereto.

[117] [Cultivated meat and Method for Producing the Same]

[118] Still another aspect of the present invention provides cultivated meat cultured using the cell scaffold according to one embodiment of the present invention.

[119] As described above, the cell scaffold according to one embodiment of the present invention is capable of self-healing within temperature and pH ranges in which cells are culturable;

accordingly, respective cell scaffolds in which myocytes and

adipocytes are cultured may be assembled to each other through a self-healing process, thereby forming marbling similar to that of actual meat.

[120] In addition, mechanical strength, such as a Young's modulus, may be readily adjusted by adjusting a mixing ratio of a chitosan resin precursor substituted with a boronic acid compound and a polymer containing a hydroxy group, by adding a strength-reinforcing material, or by repeating freezing and thawing. Accordingly, cell scaffolds having a strength favorable for culturing myocytes and a strength favorable for culturing adipocytes, respectively, may be produced, and cultivated meat in which marbling is formed may be provided by integrating the cell scaffolds through self-healing either after cell culture or before cell culture.

[121] Still another aspect of the present invention provides a method for producing cultivated meat using a cell scaffold including a self-healing hydrogel, the method including: preparing a first self-healing hydrogel and a second self-healing hydrogel, respectively, having different mixing ratios of a chitosan resin precursor substituted with a boronic acid compound and a polymer containing a hydroxy group; and [122] culturing myocytes in one of the first self-healing hydrogel and the second self-healing hydrogel, and culturing and differentiating adipocytes in the other one.

[123]

[124] In one embodiment, the first self-healing hydrogel and the second self-healing hydrogel may have different mixing ratios of a chitosan resin precursor substituted with a boronic acid compound and a polymer containing a hydroxy group. For example, the first self-healing hydrogel may be mixed so as to have a Young's modulus of 200 to 400 kPa, and the second self-healing hydrogel may be mixed so as to have a Young's modulus of 50 to 180 kPa.

[125] In one embodiment, in order to adjust mechanical strength, the method may further include freezing and then thawing the first self-healing hydrogel and the second self-healing hydrogel, and a strength-reinforcing material may be further included as needed.

[126] In one embodiment, myocytes are cultured in one of the first self-healing hydrogel and the second self-healing hydrogel, and adipocytes are cultured and differentiated in the other one, and then, the hydrogels are stacked or arranged to be in contact with each other, such that cultivated meat in which the contents of muscle tissue and adipose tissue may be adjusted to a desired ratio may be produced while the hydrogels are assembled to each other through a self-healing process.

[127] Alternatively, in a state in which the first self-healing hydrogel and the second self-healing hydrogel are stacked or arranged to be in contact with each other so as to be assembled with each other through a self-healing process, myocytes may

be cultured in one of the first self-healing hydrogel and the second self-healing hydrogel, and adipocytes may be cultured and differentiated in the other one.

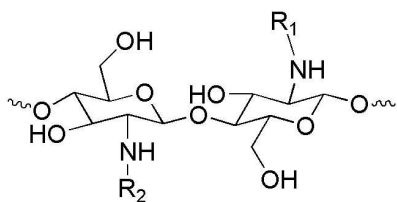
[128] The cultivated meat produced by the production method according to one embodiment may have the contents of muscle tissue and adipose tissue adjusted to a desired ratio, which may be adjusted by those skilled in the art.

[129] [Biocompatible Self-Healing Polymer]

[130] Still another aspect of the present invention provides a biocompatible self-healing polymer including a unit (A) derived from chitosan substituted with a boronic acid compound, and a unit (B) derived from a polymer containing a hydroxy group.

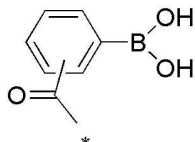
[131] In one embodiment, the unit (A) derived from chitosan substituted with a boronic acid compound may include a compound represented by the following Chemical Formula 1, but is not limited thereto.

[132] [Chemical Formula 1]

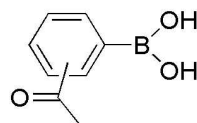


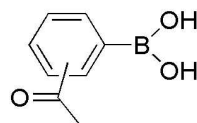
[133]

[134] In Chemical Formula 1, R_1 and R_2 are each independently



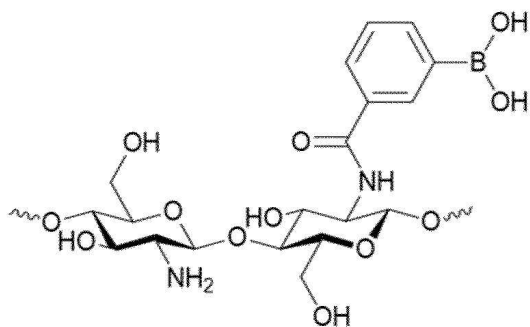
selected from hydrogen or , and



[135] at least one of R_1 and R_2 is .

[136] In one embodiment, the unit (A) derived from chitosan substituted with a boronic acid compound represented by Formula 1 may be represented by the following Chemical Formula 2, but is not limited thereto.

[137] [Chemical Formula 2]



[138]

[139] In one embodiment, the unit (A) derived from chitosan substituted with a boronic acid compound may have a substitution rate of the boronic acid compound according to the following Equation 1 of 10% or more, 15% or more, 20% or more, 25% or more, 30% or more, 35% or more, 50% or less, 45% or less, or 40% or less, and may be any value between the above values. For example, the substitution rate of the boronic acid compound may be 10 to 50%, 15 to 45%, or 20 to 30%, but is not limited thereto. Within the substitution rate satisfying the above range, unsubstituted amines of chitosan may be stabilized so as to maintain boronic acid ester bonds of the boronic acid compound. Accordingly, self-healing may be achieved.

[140] [Equation 1]

[141] Substitution rate = (Number of moles of amines substituted with boronic acid compound/Total number of moles of amines in chitosan) × 100

5 [142] In Equation 1, the total number of moles of amines of chitosan refers to the number of moles of amines present without being acetylated when chitosan is deacetylated. The total number of moles of amines of chitosan may be calculated from the content of glucosamine.

10 [143] In one embodiment, the self-healing hydrogel may include the unit (A) derived from chitosan substituted with a boronic acid compound and the unit (B) derived from a polymer containing a hydroxy group in a weight ratio of 0.1 to 0.9:1, 0.15 to 0.9:1, 0.3 to 0.9:1, or 0.6 to 0.9:1, but is not limited thereto.

15 [144] The self-healing polymers may be assembled with each other at a pH of 6.5 to 12, but are not limited thereto. In addition, self-healing may be exhibited within various temperature ranges, and from the viewpoint of biocompatibility, the self-healing polymers may be assembled with each other through a self-healing
20 process even at a biocompatible temperature. For example, the self-healing polymers may be assembled with each other through a self-healing process at 20°C or higher, 25°C or higher, 26°C or higher, 27°C or higher, 50°C or lower, 45°C or lower, 40°C or lower, 37°C or lower, or 25°C to 37°C.

25 [145] In one embodiment, the self-healing polymer may have a

dual network formed through boronic acid ester bonds and hydrogen bonds.

[146] In one embodiment, the self-healing polymer may be a hydrogel, but is not limited thereto.

5 [147] In one embodiment, the self-healing polymer may be adjusted to have a Young's modulus in a range of 50 to 400 kPa by adjusting a ratio of the unit (A) and the unit (B). The self-healing polymer is not limited thereto, provided that the above Young's modulus range is satisfied, and the unit (A) and
10 the unit (B) derived from a polymer containing a hydroxy group may be included in a weight ratio of 0.1 to 0.9:1, 0.15 to 0.9:1, 0.3 to 0.9:1, or 0.6 to 0.9:1.

[148] In one embodiment, the self-healing polymer may further include a strength-reinforcing material to adjust mechanical
15 strength. The type and content of the strength-reinforcing material are the same as described above, and thus a detailed description thereof is omitted.

[149] In one embodiment, the self-healing polymer may also be used as a tissue regeneration inducing scaffold capable of
20 inducing cell adhesion or gel degradation.

[150] In one embodiment, the self-healing polymer may be used as a carrier for delivering a bioactive substance, and the bioactive substance may be, for example, one selected from the group consisting of antibiotics, anticancer agents, analgesics,
25 anti-inflammatory agents, antiviral agents, antibacterial

agents, proteins, peptides, nucleic acids, polysaccharides, lipids, carbohydrates, steroids, extracellular matrix substances, and cells, or a mixture of two or more thereof, but is not limited thereto.

5 [151] In one embodiment, a delivery rate and the like of the bioactive substance may be controlled depending on a type and concentration of a bioactive substance delivery carrier chemically or physically bound to the self-healing polymer, and physical strength, chemical properties, and a degradation rate
10 of the self-healing polymer. The delivery carrier refers to one capable of carrying or chemically binding a bioactive substance and delivering the bioactive substance into a living body, and may be injected into the living body depending on a site of use or an administration method, or may be configured
15 to be released uniformly over a predetermined period of time at a predetermined site.

[152] As an example, the self-healing polymer may be applicable to a biocompatible dressing, a surgical-site sealing adhesive, a film, a suture, a scaffold for artificial skin, and the like,
20 but is not limited thereto.

[153] Hereinafter, the present invention will be described in more detail based on the following examples and comparative examples. However, the following examples and comparative examples are only examples for explaining the present invention
25 in more detail, and the present invention is not limited by the

following examples and comparative examples.

[154] The following physical properties were measured as described below.

[155] [Spectroscopy]

5 [156] The attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of the freeze-dried ChsB(OH)₂ resin precursor and the resin were recorded using a Nicolet iS50 FTIR spectrometer (Thermo Scientific, USA) and a Smart iTR diamond/ZnSe ATR accessory (incident angle: 45°), in a range
10 of 4,000 to 650 cm⁻¹, with a scan interval of 2 cm⁻¹ and 128 scans.

[157] The UV-Vis spectra of the ChsB(OH)₂ sample were measured using a UV-visible-near-infrared (NIR) spectrophotometer (Jasco V-770, USA). The UV data were used to confirm whether 3-carboxybenzenboronic acid was grafted onto chitosan, and to
15 calculate the degree of substitution (DS) of the boronic acid moiety according to the following equation.

$$[158] \text{ DS} = [\text{BA}] / [\text{GlnN}] \times 100$$

[159] Here, [BA] represents the content of the boronic acid moiety (mmol g⁻¹), and [GlnN] represents the content of
20 glucosamine (a chitosan repeating unit) in the sample. At this time, the average degree of deacetylation was assumed to be 80% (4.718 mmol g⁻¹).

[160] [Gel Fraction (%)]

[161] The gel fraction of the prepared self-healing hydrogel
25 (polymer) was measured from the freeze-dried weights of the

prepared self-healing hydrogel before and after immersion in ion-exchanged water.

[162] That is, the prepared self-healing hydrogel (polymer) was freeze-dried until a constant weight was obtained, and the weight was measured and designated as W_i . Next, the hydrogel was immersed in ion-exchanged water until a constant weight was obtained, removed, and then freeze-dried again until a constant weight was obtained, and the weight was measured and designated as W_e .

10 [163] The gel fraction was calculated according to the following equation.

$$[164] \text{ Gel Fraction} = (W_e/W_i) \times 100$$

[165] [Swelling Degree]

[166] In order to determine the swelling degree, the self-healing hydrogel (polymer) was weighed to measure its weight (W_d), and the resin was immersed in an excess of ion-exchanged water at room temperature and allowed to swell until equilibrium was reached. Thereafter, the swollen resin was weighed to measure the weight of the resin in a swollen state (W_s), and the equilibrium swelling degree (ESD) was determined according to the following equation.

$$[167] \text{ ESD (\%)} = (W_s - W_d)/W_d \times 100\%$$

[168] [Mechanical Properties]

[169] The compressive mechanical properties of the prepared self-healing hydrogel (resin) samples were evaluated at room

temperature using a universal testing machine (UTM, Instron 5943, UK) equipped with a 1 kN load cell. The resin samples were cut into cubes having dimensions of 0.5 cm × 0.5 cm × 0.5 cm and placed on a support plate. The compression test was performed at a crosshead speed of 5 mm/min. Data were collected at intervals of 0.1 seconds, and Young's modulus was defined as the compressive elastic modulus (E) and was determined from the slope of the linear region of the stress-strain curve. In addition, the compressive modulus was measured at strain values of 30% and 50%, respectively.

[170] [Response of Resin to Changes in Glucose Concentration and pH]

[171] The stabilized resin was exposed to an acidic pH by adding an HCl solution (0.1 M) to dissociate the boronic acid-diol ester bonds. The pH was increased again to 7 to 8 using an NaOH solution (0.1 M) to reform the boronic acid-diol ester bond network. In addition, the response of the resin to aqueous glucose solutions (0.45 wt% and 5 wt%) was also investigated. Glucose, which is a saccharide having a polyol group, competes with boronic acid-diol ester bonds and is an essential component of mammalian cell culture media (used at 0.45 wt%). It was expected that a low glucose concentration would not cause significant disruption of the resin.

[172] [Meat Cultured on Resin-Based Scaffold]

[173] 1. Cell Lines and Cell Treatment

[174] 3T3-L1 is a mouse fibroblast cell line that may be differentiated into adipocytes. C2C12 is a mouse myoblast cell line that may be rapidly differentiated to form contractile myotubes and produce muscle proteins. Both cell lines were
5 obtained from ATCC (<https://www.atcc.org/>) and were handled in accordance with the guidelines provided by the supplier.

[175] 2. Cell Viability and Proliferation
(Biocompatibility/Cytotoxicity of Resin Scaffold)

[176] The cytotoxicity of ChSB(OH)₂ and PVA, which are resin
10 components, was evaluated using a Cell Counting Kit-8 (CCK-8) based on ISO-10993-5 and ISO-10993-5-12 standards with C2C12 myoblasts and 3T3-L1 fibroblasts. Freeze-dried test materials were added to cell culture medium at 0.2 g mL⁻¹ and incubated at 37°C under shaking at 100 rpm for 24 hours, and then the
15 extracts were tested on the target cell lines. The growth medium and extracts of low-density polyethylene (LDPE, 0.2 g mL⁻¹) and zinc diethyldithiocarbamate (100 µM) were used as blank, negative, and positive controls, respectively. Cells were cultured in polystyrene 96-well cell plates at 10⁴ cells
20 mL⁻¹ and allowed to proliferate in the growth medium for 24 hours, and then the cells were incubated with the extracts for 24 hours. Thereafter, the cells were washed three times with phosphate-buffered saline (PBS), and in Dulbecco's Modified Eagle Medium (DMEM) containing 10 v% of Cell Counting Kit-8
25 (CCK-8), at 37°C for 1 hour, intracellular mitochondrial

dehydrogenases converted the tetrazolium salt of CCK-8 into an orange-colored formazan, which was released from the cells into the medium. The amount of formazan was measured using a microplate reader (Hidex Sense) at an absorbance of 450 nm (optical density, OD 450). The cell viability was compared with that of the blank using the following equation.

$$[177] \text{ Viability (\%)} = (100\% \times \text{OD}_{450\text{e}}) / \text{OD}_{450\text{b}}$$

[178] Here, $\text{OD}_{450\text{e}}$ is the measured OD 450 value of the extract of the test sample, and $\text{OD}_{450\text{b}}$ is the measured OD 450 value of the blank.

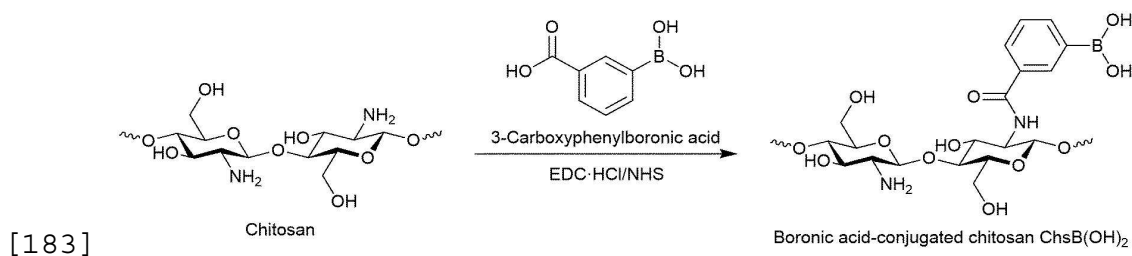
[179] In addition, cell proliferation on the resin scaffold was monitored for one week using CCK-8. The cells were seeded at 10^6 cells mL^{-1} onto a resin scaffold having a height of 0.5 cm in a 24-well cell plate that had been pre-equilibrated with growth medium (cell culture medium for cell proliferation). 500 μL of the growth medium was added to the top of the resin scaffold and was refreshed daily to maintain healthy cell growth and proliferation. The OD 450 values were measured in the same manner as described above. However, prior to measurement, the reacted CCK-8 solution was transferred from the culture plate to a new 96-well cell plate to prevent interference from the resin scaffold. Thereafter, fresh growth medium was added to the cell culture medium to continue the experiment. An increase in the OD 450 value over time indicates cell proliferation.

[180] The cell viability was visually confirmed using a

LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells (Thermo Fisher Scientific, L-3224). The cells cultured on the resin scaffold (10^6 cells mL^{-1}) were washed three times with PBS, and then stained with calcein acetoxymethyl ester (calcein AM, for staining live cells; $2 \mu\text{M}$ in PBS) and ethidium homodimer-1 (EthD-1, for staining dead cells; $4 \mu\text{M}$ in PBS) at 37°C for 30 minutes in the dark. Thereafter, cell nuclei were stained with Hoechst 33342 ($5 \mu\text{g mL}^{-1}$ in PBS) at 37°C for 5 minutes in the dark, and then washed once with PBS. The stained cells were observed using a laser scanning fluorescence microscope (LSM 780, Carl Zeiss AG, Germany) under the following parameters: calcein AM, excitation at 495 nm and emission at 515 nm (green); EthD-1, excitation at 528 nm and emission at 617 nm (red); and Hoechst 33342, excitation at 350 nm and emission at 461 nm (blue).

[181] The cell viability and proliferation were compared with those of Corning Matrigel Matrix (product No. 354230, 5 mg mL^{-1}), which mimics the extracellular matrix and serves as a positive control.

[182] [Preparation Example 1] Synthesis of Boronic Acid-Substituted Chitosan (Resin Precursor)



[184] Chitosan (5.0 g; Sigma-Aldrich, medium molecular weight chitosan, product No. 448877, viscosity molecular weight M_v of 190 to 310 kDa, 0.0236 mol on a glucosamine monomer unit basis, a degree of deacetylation of 80%) was dissolved in ion-exchanged water (500 mL). The pH of the solution was adjusted to 4 to 4.5 using HCl (0.1 M). 3-Carboxybenzeneboronic acid (1.958 g, 0.0118 mol, corresponding to 50 mol% relative to glucosamine) was added to the chitosan solution. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (2.262 g, 0.0118 mol) and N-hydroxysuccinimide (NHS) (1.358 g, 0.0118 mol) as crosslinkers were added to the reaction mixture, and the reaction was allowed to proceed at room temperature for 48 hours. The boronic acid-bound chitosan [ChsB(OH)₂] was purified using a regenerated cellulose dialysis membrane having a molecular weight of 10 KDa (Spectra/Por™, Spectrum Labs), and was preserved by freeze-drying.

[185] As a result, as illustrated in FIG. 1, successful grafting of 3-carboxybenzeneboronic acid onto chitosan was confirmed by FTIR spectroscopy. The spectrum of ChsB(OH)₂ exhibited characteristic phenyl group signals, including aromatic ring stretching (1,580 cm⁻¹) and out-of-plane deformation of three adjacent CH bonds of an m-substituted aromatic ring (748 and 705 cm⁻¹). In addition, the formation of an amide bond in ChsB(OH)₂ was confirmed by a C=O stretching vibration (1,604 cm⁻¹).

[186] The bonding was also confirmed by UV-Vis spectroscopy as shown in FIG. 2, and, as illustrated in FIG. 2, the appearance of a phenyl ring peak at 285 nm was observed. In addition, the degree of boronic acid substitution was determined by ultraviolet-visible spectroscopy (UV-Vis spectroscopy) based on a calibration curve for 3-carboxybenzenboronic acid, as shown in FIG. 3. As a result, the degree of substitution was determined to be 21.2 mol%.

[187] [Comparative Example 1] Synthesis of Alginate-Based Self-Healing Resin Receptor for Cell Culture

[188] $\text{AlgB}(\text{OH})_2$, in which boronic acid was bound to alginate, was prepared by grafting 3-aminophenylboronic acid onto alginate in the presence of $\text{EDC} \cdot \text{HCl}$ and NHS, as described below. Sodium alginate (5 g, 0.025 mol on a monomer unit basis) was dissolved in ion-exchanged water (500 mL), and $\text{EDC} \cdot \text{HCl}$ (4.8 g, 0.025 mol), NHS (2.88 g, 0.025 mol), and 3-aminophenylboronic acid (1.95 g, 0.0125 mmol) were added. The mixture was stirred at room temperature for 48 hours, and was then dialyzed against distilled water for one week, during which the water was replaced at least seven times, and subsequently freeze-dried.

[189] $\text{AlgB}(\text{OH})_2$ was able to form a stable resin with PVA only at an alkaline pH of 10, regardless of the ratio between the two components. At a physiological pH of 7 to 7.5, the resin began to exhibit fluidity and rapidly transformed into a flowable liquid.

[190] [Examples 1 to 10]

[191] **<Synthesis of Boronic Acid Ester-Based Self-Healing Hydrogel Resin>**

[192] The freeze-dried $\text{ChsB}(\text{OH})_2$ prepared in Preparation
5 Example 1 was dissolved at 3 wt% in an aqueous acetic acid
solution (0.2 M, pH 4.76) (Solution A). Polyvinyl alcohol (PVA)
powder ($\text{MW } 89 \times 10^3$ to 98×10^3 g/mol, > 99 mol% hydrolyzed,
atactic hydroxy side chains) was dissolved in deionized water
at 10 wt% and 90°C (Solution B). In Examples 3 and 8,
10 spray-dried cellulose nanocrystals were additionally added at
0.1 wt% to the compositions of Example 2 and Example 7,
respectively.

[193] In order to form a hydrogel, the solution A was added to
the solution B at different mass ratios as shown in Table 1,
15 and crosslinking was induced by adjusting the pH to 7.5 using
a 1.0 M NaOH solution.

[194] In order to remove air bubbles trapped in the resin and
to obtain a uniform texture, the resin was mixed using a mixer,
and then stabilized at 4°C for 24 hours to induce complete
20 crosslinking of the polymer network. Examples 6 to 10 were
subjected to a process in which the resin was placed in a freezer
at -20°C for 6 hours and then thawed at 20°C for 12 hours in
order to improve mechanical properties. This process induces
additional crosslinking through hydrogen bonding between PVA
25 chains in the resin, thereby forming a dual network in the resin

through boronic acid ester bonds and hydrogen bonds. The resin was purified by dialysis using a regenerated cellulose membrane having a molecular weight of 10 KDa against ion-exchanged water and Dulbecco's phosphate-buffered saline (PBS 1X, pH 7.4, for cell culture) until by-products were completely removed.

[195] <Evaluation of Gel Fraction and Swelling Degree>

[196] In Table 1, the sample designation N indicates a sample that was not subjected to a freeze-thaw treatment, F indicates a sample to which a freeze-thaw process was additionally applied, and C indicates a sample in which cellulose nanocrystals were added as a strength-reinforcing agent in an amount of 0.1 wt% based on the total hydrogel resin. The numerical values represent the weight ratio of ChsB(OH)₂ relative to PVA.

[197] The physical properties of the hydrogel resins prepared in Examples 1 to 10 were evaluated.

[198] [Table 1]

	Sample	ChsB(OH) ₂ /PVA mass ratio	Gel fraction (%)	Equilibrium degree of swelling (%)
Example 1	F0.5	0.15	75.6 ± 2.59	145 ± 2.52
Example 2	F1	0.3	83.1 ± 3.30	153 ± 7.57
Example 3	F1C	0.3	84.4 ± 1.20	152 ± 1.73
Example 4	F2	0.6	64.6 ± 4.65	188 ± 6.66
Example 5	F3	0.9	57.7 ± 3.18	196 ± 3.61
Example 6	N0.5	0.15	66.9 ± 2.23	201 ± 5.13
Example 7	N1	0.3	73.9 ± 2.73	182 ± 4.16
Example 8	N1C	0.3	77.1 ± 1.81	185 ± 6.03
Example 9	N2	0.6	63.8 ± 3.94	231 ± 6.03
Example 10	N3	0.9	51.8 ± 2.65	237 ± 5.86

[199] As can be seen from Table 1, the ChsB(OH)₂ solution and

the PVA solution were mixed at various volume ratios within a physiological pH range of 7.0 to 7.5, and it was confirmed that a self-healing hydrogel was formed through boronic acid-diol bonding. Such bonding is stabilized by nitrogen atoms of chitosan (N series). The freeze-thaw treatment induces hydrogen bonding between PVA chains, thereby mechanically reinforcing the hydrogel (F series). <Evaluation of Mechanical Properties>

[200] In addition, as can be seen from Table 2, the formation of a hydrogen-bonding network between PVA chains was reflected in a high gel fraction and a low swelling degree.

[201] [Table 2]

	Sample	Compressive modulus (MPa)	At 30% strain Stress (MPa)	At 50% strain Stress (MPa)
Example 1	F0.5	0.16	0.10	0.39
Example 2	F1	0.42	0.22	0.92
Example 3	F1C	0.61	0.34	1.60
Example 4	F2	0.10	0.07	0.23
Example 5	F3	-	-	-
Example 6	N0.5	0.08	0.05	0.26
Example 7	N1	0.21	0.13	0.47
Example 8	N1C	0.25	0.15	0.17
Example 9	N2	0.07	0.04	0.37
Example 10	N3	-	-	-

[202] As shown in Table 2, F3 of Example 5 and N3 of Example 10 were not sufficiently strong to measure mechanical properties and therefore could not be measured. In addition, as can be seen from the table, the compressive strength properties of the hydrogel could be varied by adjusting the mass ratio of ChsB(OH)₂ to PVA. This capability is advantageous for

promoting tissue growth and differentiation by generating a scaffold having an elastic modulus that matches a specific type of tissue. The compressive elastic modulus exhibited the highest value when ChsB(OH)₂ and PVA were mixed at an appropriate mass ratio of 0.3.

[203] <Evaluation of Self-Healing and Multi-Module Responsiveness>

[204] Next, self-healing and multi-module responsiveness were evaluated.

10 [205] The dynamic nature of the boronic acid-diol bonds allows efficient self-healing of the ChsB(OH)₂/PVA hydrogel and leads to efficient fusion of incompatible interfaces. As shown in FIG. 4, three separately prepared specimens of the N1 hydrogel of Example 7, which were independently prepared and dyed
15 (stained with Brilliant Blue FCF/unstained/stained with Amaranth Red 2), were brought into surface-to-surface contact with each other, and after 5 minutes, the three separate pieces were smoothly connected and transformed into a hydrogel that could be lifted without separation of the pieces (see FIG. 4a).

20 [206] Such stitching-healing functionality enabled easy shape reconfiguration of the hydrogel. For example, a piece of the N1 hydrogel shaped as a teddy bear head was cut and completely fragmented, and then placed into molds having heart, star, and flower shapes; after a certain period of time, it was observed
25 that the hydrogel was re-formed into heart-, star-, and

flower-shaped structures corresponding to the respective molds. In this process, no additional adhesive was required, and it was confirmed that the self-healing polymer was reusable (see FIG. 4b).

5 [207] The N1 hydrogel also responded to changes in pH and to glucose. When the pH was lowered from alkaline to acidic conditions, the hydrogel collapsed due to dissociation of pH-dependent boronic acid-diol bonds between ChsB(OH)₂ and PVA chains. When the pH was increased again to 7.5, the gel was
10 re-formed and recovered its stability (see FIG. 4c).

[208] In addition, when the N1 hydrogel was brought into contact with a 5 wt% glucose solution, the gel began to flow and soon exhibited free fluidity due to strong competitive binding between the monosaccharide and boronic acid. However, when the
15 hydrogel was placed in DMEM medium having a much lower glucose concentration (0.45 wt%), the hydrogel was not damaged, which may provide properties essential for functioning as a scaffold for tissue loading (see FIGS. 4d and 4e).

[209] **<Evaluation as Scaffold for Tissue Culture>**

20 [210] Next, the feasibility of using the self-healing hydrogel as a material for a scaffold for tissue culture was evaluated. Cell culture of cell lines was performed using the hydrogels prepared in Examples 1 to 10.

[211] The cell line culture was evaluated using a Cell Counting
25 Kit-8 (CCK-8) by examining the growth and proliferation of one

cell line on hydrogels containing different cell lines. In this experiment, for each individual cell line, a hydrogel exhibiting a proliferation rate similar to that of Matrigel (positive control) was selected. The following hydrogels and
5 the corresponding compressive strengths were used: for the C2C12 cell line, N1 (207 kPa) and N1C (250 kPa) were used, and for the 3T3-L1 cell line, N0.5 (80.2 kPa), F0.5 (161 kPa), and F2 (104 kPa) were used.

[212] First, cells were seeded onto a pre-equilibrated hydrogel
10 (height of about 0.3 cm) in a 24-well plate using a growth medium at 10^6 cells mL⁻¹. Thereafter, in order to facilitate cell attachment, a growth medium (500 μ L) was added onto the top of the seeded hydrogel, and then incubated at 37°C under 5% CO₂ for 3 hours. After aspirating the medium, the C2C12-seeded
15 hydrogel was carefully detached from the well using a spatula and inverted, and then attached to a 3T3-L1-seeded hydrogel in another well plate by utilizing the self-healing capability of the hydrogel. Six self-healing hydrogel combinations were evaluated. The cell culture was performed by adding a growth
20 medium (500 μ L) and incubating at 37°C under 5 v% CO₂, and the medium was replaced daily to maintain optimal cell growth and proliferation. In consideration of the slower growth rate of the 3T3-L1 cell line compared to the C2C12 cell line, the growth medium was supplemented with bovine calf serum (BCS, 10 v%),
25 which is optimized for the growth of 3T3-L1 fibroblasts. In

co-culture, the growth and proliferation of the two cell lines were evaluated using CCK-8 in the same manner as described for individual cell culture. Matrigel (5 mg mL⁻¹), which lacks self-healing capability, was used as a negative control.

5 [213] 1. Cell Viability and Proliferation

[214] All hydrogel components composed of ChsB(OH)₂ and PVA exhibited no observable cytotoxicity in the 3T3-L1 and C2C12 cell lines. The cell viability under all treatment conditions complied with the ISO 10993-5 standard and was comparable to
10 that of the negative control (see FIG. 5).

[215] In addition, the effect of hydrogel stiffness on cell proliferation was investigated. Soft hydrogels (F0.5, N0.5, and F2) promoted the proliferation of the 3T3-L1 fibroblast cell line prior to differentiation (see FIG. 6), whereas the C2C12
15 myoblast cell line grew better on stiffer hydrogels (N1 and N1C) (see FIG. 7). On these hydrogels, the growth of both cell lines was comparable to that on Matrigel (positive control).

[216] According to the LIVE/DEAD staining results, the viability of 3T3-L1 cultured on the F0.5 hydrogel and the
20 viability of C2C12 cultured on the N1 hydrogel were almost identical to those of Matrigel, which is a positive control (see FIG. 8). That is, it can be seen that there were almost no dead cells. In Table 7, green indicated by ① (calcein AM) represents live cells, and blue indicated by ② (Hoechst 33342) represents
25 cell nuclei.

[217] 2. Preparation of Differentiation Media for C2C12 and 3T3-L1 Cell Lines and Differentiation

[218] Next, cell differentiation was examined.

[219] In order to differentiate 3T3-L1 cells into adipocytes, a differentiation medium having the following composition was prepared: 90% DMEM, 10% FBS, 1 uM dexamethasone, 0.5 mM methyl isobutyl xanthine (IBMX), and 1 ug/ml insulin. After removal of the growth medium from sufficiently grown 3T3-L1 cells, the differentiation medium was applied. Depending on the day of observation, the differentiation medium was applied for at least 7 days and up to 14 days or 21 days, and the differentiation medium was refreshed every 2 to 3 days.

[220] Similarly, in order to differentiate C2C12 cells into myoblasts, a differentiation medium having the following composition was prepared: 98% DMEM, 2% donor equine serum (DES), and 1 uM insulin. After removal of the growth medium from sufficiently grown C2C12 cells, the differentiation medium was applied. Depending on the day of observation, the differentiation medium was applied for at least 7 days and up to 14 days or 21 days, and the differentiation medium was refreshed every 2 to 3 days.

[221] 3T3-L1 cells differentiated into adipocyte-like tissues in which lipid droplets stained with Nile Red were accumulated on the F0.5 hydrogel and on Matrigel. Similarly, C2C12 cells underwent a morphological change from fibroblast-like

elongated shapes to myocyte-like shapes on the N1 hydrogel and Matrigel (see FIG. 9). That is, differentiation of 3T3-L1 into adipose-like tissues on the F0.5 hydrogel and Matrigel, and differentiation of C2C12 into muscle-like tissues on the N1 hydrogel and Matrigel, were observed. The cells were observed on day 9 of differentiation using a fluorescence microscope. Red (Nile Red) indicated by ① represents lipid droplets, blue (Hoechst 33342) indicated by ② represents cell nuclei, and green (Alexa Fluor 488) indicated by ③ represents myosin and F-actin.

[222] 3. Production of Cultivated meat

[223] Adipose (3T3-L1) and muscle (C2C12) tissue cultures were cultured in 6-well culture plates to produce an artificial meat prototype. The hydrogel cell scaffold had a thickness not exceeding 0.3 cm and was prepared by adjusting its size to fit the well, mixing ChsB(OH)₂ (3 wt%, pH of 4 to 5) and a PVA (10 wt%) solution at an appropriate ratio, and adjusting the pH to 7.4 to 7.5. A freeze-thaw treatment may be applied to the hydrogel. 24 hours prior to cell seeding, the scaffold was impregnated with a growth medium appropriate for each cell type to establish an equilibrium state.

[224] The cells (1 mL of a suspension at 10⁶ cells/mL) were seeded onto the hydrogel scaffold in each well (N1 for C2C12 and F0.5 for 3T3-L1). The cells were cultured until reaching 100% confluence to ensure sufficient intercellular signaling for differentiation.

[225] The confluent 3T3-L1 cells were induced to undergo adipogenic differentiation for 3 days using an adipocyte differentiation medium, and then were maintained in an adipocyte maintenance culture medium that was freshly replaced
5 daily. The confluent C2C12 cells were induced to undergo myogenic differentiation and were maintained by daily replacement of the culture medium. The compositions of the growth, differentiation, and maintenance media are described in "2. Preparation of Differentiation Media for C2C12 and 3T3-L1
10 Cell Lines and Differentiation".

[226] After 28 days of differentiation, mature adipose and muscle tissue cultures were used to assemble a meat prototype through scaffold self-healing. After removal of the culture medium, the adipose and muscle layers were arranged side by side
15 and assembled into a sandwich structure (FIG. 10), a bacon/pork belly-like structure by aligning the edges, or customized structures mimicking complex marbled beef. The assembly was completed within 2 to 5 minutes at room temperature without the use of any external adhesive or stimulus. The assembled meat
20 was confirmed to be capable of being stored under refrigerated conditions or cooked as desired.

[227] The differentiated N1 muscle-like culture and F0.5 adipose-like culture exhibited an appearance similar to that of muscle and fat of actual raw meat after 28 days (see FIG.
25 10a). The muscle tissue and adipose tissue were distinguished

using carmine, which is a common food coloring agent used to impart a red color to processed meat. The two cultures were fried in sunflower oil at 150°C, and as a result, the cooked artificial meat exhibited a texture similar to that of beef jerky or bacon. Finally, by alternately stacking cell-laden hydrogel sheets to a thickness of up to 0.75 µm, a thick meat piece (thickness of 0.35 cm), in which customized patterns were formed within adipose and muscle structures, could be produced (see FIG. 10b). This demonstrates the potential to produce large-area artificial meat without the assistance of advanced technologies such as 3D printing.

[228] <Results of Confirmation of Edibility and Stability>

[229] 1. Evaluation of Hydrogel Weight

[230] For compositional analysis of the tissue cultures, the weights of the N1 and F0.5 hydrogels that had reached a fully equilibrated state were measured prior to cell seeding. On day 28 after differentiation, the culture media were removed from the corresponding cultures (N1 containing C2C12 cells and F0.5 containing 3T3-L1 cells), the cultures were washed with distilled water, surface moisture was removed, and the weight (W_s) was measured. Thereafter, the cultures were freeze-dried to obtain dried contents, and the weight (W_d) of the dried hydrogel was measured. The dried tissue contents were obtained by subtracting the weights of the dried hydrogel and water from the total weight of the cultures, assuming that no degradation

of the hydrogel occurred during the culture period. The equilibrium swelling degree (ESD) was calculated according to the following equation.

$$[231] \text{ ESD (\%)} = (W_s - W_d) / W_d \times 100\%$$

5 [232] 2. Compositional Analysis of Hydrogel

[233] Elemental analysis of the dried contents of the cultures was performed using a Unicube elemental analyzer (Elementar, Germany) for hydrogen (H), carbon (C), nitrogen (N), and sulfur (S), and a Flash 2000 elemental analyzer (Thermo Fisher Scientific, USA) for oxygen (O). For analysis of boron content, a freeze-dried sample (to 0.1 g) was ground in liquid nitrogen and then initially dispersed in distilled water (100 mL) by sonication (40% amplitude, 5 s on/2 s off cycle, at room temperature, for 3 minutes). Thereafter, the dispersion was
10 analyzed using an iCAP 6500 Duo inductively coupled plasma-atomic emission spectrometer (ICP-AES).

[234] The experimental results are shown below.

[235] After differentiation, the compositions of the 3T3-L1/F0.5 culture as an adipocyte/cell scaffold and the
20 C2C12/N1 culture as a myocyte/cell scaffold were measured.

[236] As a result of confirming the weight composition of the differentiated 3T3-L1/F0.5 culture, it was confirmed that the culture was composed of 19.5% moisture, 9.68% dried hydrogel, and 70.84% dried tissue. Similarly, as a result of confirming
25 the weight composition of the differentiated C2C12/N1 culture,

it was confirmed that the culture was composed of 73.29% moisture, 4.82% dried hydrogel, and 21.89% dried tissue.

[237] It was confirmed that adipose tissue, which stores neutral lipids, contains less water than muscle tissue, whereas muscle tissue retains a water content that is important for electrolyte transport and contraction. After 28 days of differentiation, the dried hydrogel contents of the C2C12/N1 and 3T3-L1/F0.5 cultures were calculated to be 4.82 wt% and 9.65 wt%, respectively. This demonstrated that, as the contents of cells and tissues increased during the processes of cell proliferation and differentiation, the volume occupied by the hydrogel gradually decreased.

[238] In addition, as a result of measuring the elemental composition of the final cultures including the scaffold and tissues, it was confirmed that the elemental composition was significantly altered after cell growth and differentiation as compared with the initial hydrogel. The results are shown in Table 3.

[239] Table 3 shows the elemental composition of the dried samples of the hydrogel precursor, the hydrogel, and the tissue cultures (cell-laden hydrogels). The unit is wt%.

[240] [Table 3]

Dried sample	Formula/Composition	Hydrogen (H)		Carbon (C)		Nitrogen (N)		Oxygen (O)		Sulfur (S)	
		Theoretical value	Experimental value	Theoretical value	Experimental value	Theoretical value	Experimental value	Theoretical value	Experimental value	Theoretical value	Experimental value
Chito	$(C_6H_{11}O_4N)_{0.8}(C_8H_{13}O_5)$	6.78	6.52	45.33	45.27	8.26	8.44	39.63	39.53	0	ND

san	N) _{0.2} ^{b)}										
PVA	C ₂ H ₄ O	9.15	8.59	54.53	54.27	0	ND	36.32	36.31	0	ND
ChsB(OH) ₂	((C ₁₃ H ₁₆ O ₇ NB) _{0.1696} (C ₆ H ₁₁ O ₄ N) _{0.6304} (C ₈ H ₁₃ O ₅ N) _{0.2} ^{c)}	6.34	6.48	46.82	46.95	7.2	6.85	38.7	37.85	0	ND
N1 hydrogel	ChsB(OH) ₂ /PVA weight ratio 0.3/0.7	7.31	7.13	49.48	48.5	4.71	5.02	37.88	37.67	0	ND
F0.5 hydrogel	ChsB(OH) ₂ /PVA weight ratio 0.15/0.85	7.92	7.85	51.15	51.82	3.15	3.12	37.36	38.03	0	ND
C2C12/N1 culture ^{d)}		-	10.00	-	19.60	-	3.44	-	66.00	-	0.25
3T3-L1/F0.5 culture ^{d)}		-	7.16	-	61.74	-	0.87	-	29.69	-	ND

[241] In Table 3, ^{b)} is based on a degree of deacetylation of 80 mol%, ^{c)} is based on an average boronic acid-N substitution degree of 21.2 mol% as determined by ultraviolet-visible spectroscopy, and ^{d)} is a sample after 28 days of cell differentiation. The 3T3-L1/F0.5 culture exhibited an increased carbon content as adipocytes accumulated long-chain hydrocarbon triglycerides, whereas the C2C12/N1 culture showed higher nitrogen and oxygen contents derived from muscle proteins. As a result of the calculation, it was shown that the elemental compositions of the differentiated C2C12 and 3T3-L1 closely matched those of myocytes and adipocytes in the database (see Table 4). This suggests that the self-healing hydrogel capable of dynamic bonding may promote normal development of muscle and adipose tissues. As a result of safety evaluation of the meat prototype based on boron content, the wet cultures cultured for 28 days contained less than 0.07 wt% of boron (corresponding to a boronic acid equivalent of 0.4

wt%). This satisfies the maximum usage requirement for boronic acid as a food additive, thereby suggesting the potential for safe consumption of the meat product.

[242] Table 4 shows the calculated elemental composition of dried muscle and adipose tissues after 28 days of differentiation. The composition is expressed in wt%.

[243] [Table 4]

Tissue	Hydrogen (H)		Carbon (C)		Nitrogen (N)		Oxygen (O)		Phosphorus (P)		Sulfur (S)	
	Db ^{a)}	Calc ^{b)}	Db	Calc	Db	Calc	Db	Calc	Db	Calc	Db	Calc
Muscle (C2C12)	10.15	10.63	13.13	13.24	3.25	3.1	72.4	72.24	0.2	0	0.33	0.3
Adipose (3T3-L1)	7.14	7.06	62.38	63.09	0.6	0.56	29.34	28.55	0.06	0	0.06	0

[244] In Table 4, ^{a)}Db is based on element% data obtained from the IT'IS Foundation database (<https://itis.swiss/virtual-population/tissue-properties/database/elements/>) for various tissue types, from which the values were calculated and expressed in wt%. ^{b)}Calc is a value calculated using the weight ratio of the tissue in the dried content. Although the present invention has been described with reference to specific matters and limited embodiments, they have been provided only for assisting in the overall understanding of the present invention. Therefore, the present invention is not limited to the embodiments, and various modifications and changes may be made by those skilled in the art to which the present invention pertains from this description.

[245] Therefore, the spirit of the present invention should not be limited to the described embodiments, but the claims and all modifications equal or equivalent to the claims are intended to fall within the spirit of the present invention.

【CLAIMS】**【Claim 1】**

A cell scaffold for producing cultivated meat, the cell scaffold comprising a self-healing hydrogel,

5 wherein cross-sections of at least two self-healing hydrogels that are identical to or different from each other are capable of being assembled with each other by forming reversible physicochemical bonds through a self-healing process.

10 **【Claim 2】**

The cell scaffold of claim 1, wherein the cell scaffold for producing cultivated meat includes a first self-healing hydrogel in which myocytes are cultured and a second self-healing hydrogel in which adipocytes are cultured.

15 **【Claim 3】**

The cell scaffold of claim 1, wherein the self-healing hydrogel forms reversible physicochemical bonds through a self-healing process in one or more environments selected from temperature and pH ranges in which cells are culturable.

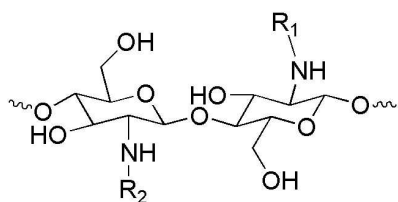
20 **【Claim 4】**

The cell scaffold of claim 1, wherein the self-healing hydrogel includes a unit (A) derived from chitosan substituted with a boronic acid compound, and a unit (B) derived from a polymer containing a hydroxy group.

25 **【Claim 5】**

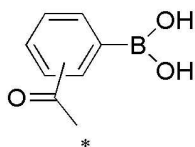
The cell scaffold of claim 4, wherein the unit (A) derived from chitosan substituted with a boronic acid compound includes a compound represented by the following Chemical Formula 1:

[Chemical Formula 1]



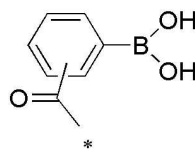
5

in Chemical Formula 1, R_1 and R_2 are each independently



selected from hydrogen or

, and



at least one of R_1 and R_2 is

.

【Claim 6】

10

The cell scaffold of claim 5, wherein the unit (A) derived from chitosan substituted with a boronic acid compound has a substitution rate of the boronic acid compound according to the following Equation 1 of 10 to 50%:

[Equation 1]

15

Substitution rate = (Number of moles of amines substituted with boronic acid compound/Total number of moles of amines in chitosan) $\times$ 100.

【Claim 7】

The cell scaffold of claim 1, wherein the self-healing hydrogel has a gel fraction of 50 to 90%.

20

【Claim 8】

The cell scaffold of claim 1, further comprising a strength-reinforcing material.

【Claim 9】

5 The cell scaffold of claim 1, wherein the cell scaffold is capable of culturing one or two or more cells selected from the group consisting of myoblasts, adipocytes, fibroblasts, myocytes, mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), satellite cells, endothelial cells, and
10 embryonic stem cells.

【Claim 10】

The cell scaffold of claim 2, wherein a first component of the self-healing hydrogel in which myocytes are cultured (proliferated) has a Young's modulus of 200 to 400 kPa, and a
15 second component of the self-healing hydrogel in which adipocytes are cultured (proliferated) has a Young's modulus of 50 to 180 kPa.

【Claim 11】

Cultivated meat cultured using the cell scaffold for
20 producing cultivated meat of any one of claims 1 to 10.

【Claim 12】

The cultivated meat of claim 11, wherein the cultivated meat has marbling similar to that of actual meat.

【Claim 13】

25 A method for producing a cell scaffold using a

self-healing hydrogel, the method comprising mixing a chitosan resin precursor substituted with a boronic acid compound and a polymer containing a hydroxy group, and then inducing crosslinking by adjusting a pH.

5 **【Claim 14】**

The method of claim 13, further comprising, after the mixing, freezing and then thawing the mixture.

【Claim 15】

The method of claim 13, wherein during the mixing, a
10 strength-reinforcing material is further added.

【Claim 16】

A method for producing cultivated meat using a cell scaffold including a self-healing hydrogel, the method comprising:

15 preparing a first self-healing hydrogel and a second self-healing hydrogel, respectively, having different mixing ratios of a chitosan resin precursor substituted with a boronic acid compound and a polymer containing a hydroxy group; and
culturing myocytes in one of the first self-healing
20 hydrogel and the second self-healing hydrogel, and culturing and differentiating adipocytes in the other one.

【Claim 17】

The method of claim 16, wherein in the preparing of the first self-healing hydrogel and the second self-healing
25 hydrogel, a strength-reinforcing material is further added.

【Claim 18】

The method of claim 16, further comprising, in the preparing of the first self-healing hydrogel and the second self-healing hydrogel, freezing and then thawing the first
5 self-healing hydrogel and the second self-healing hydrogel.

【Claim 19】

The method of claim 16, wherein the first self-healing hydrogel has a Young's modulus of 200 to 400 kPa, and the second self-healing hydrogel has a Young's modulus of 50 to 180 kPa.

10 **【Claim 20】**

The method of claim 16, wherein the first self-healing hydrogel and the second self-healing hydrogel are assembled with each other through a self-healing process by culturing myocytes in one of the first self-healing hydrogel and the
15 second self-healing hydrogel, culturing and differentiating adipocytes in the other one, and then stacking the self-healing hydrogels or arranging the self-healing hydrogels to be in contact with each other.

【Claim 21】

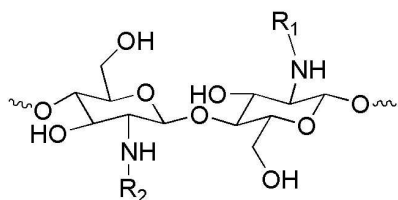
20 The method of claim 16, wherein in a state in which the first self-healing hydrogel and the second self-healing hydrogel are stacked or arranged to be in contact with each other so as to be assembled with each other through a self-healing process, myocytes are cultured in one of the first self-healing
25 hydrogel and the second self-healing hydrogel, and adipocytes

are cultured and differentiated in the other one.

【Claim 22】

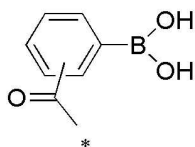
A biocompatible self-healing polymer comprising a unit (A) derived from chitosan to which a boronic acid compound including a compound represented by the following Chemical Formula 1 is bonded, and a unit (B) derived from a polymer containing a hydroxy group:

[Chemical Formula 1]

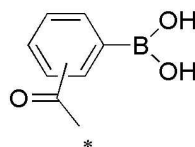


in Chemical Formula 1, R_1 and R_2 are each independently

selected from hydrogen or



, and



at least one of R_1 and R_2 is

【Claim 23】

The self-healing polymer of claim 22, wherein the self-healing polymers are assembled with each other at a pH of 6.5 to 12.

【Claim 24】

The self-healing polymer of claim 22, wherein the unit (A) derived from chitosan substituted with a boronic acid compound has a substitution rate of the boronic acid compound

according to the following Equation 1 of 10 to 50%:

[Equation 1]

Substitution rate = (Number of moles of amines substituted
with boronic acid compound/Total number of moles of amines in
5 chitosan) × 100.

【Claim 25】

The self-healing polymer of claim 22, further comprising
a strength-reinforcing material.

【Claim 26】

10 The self-healing polymer of claim 22, wherein the
self-healing polymer has a Young's modulus of 50 to 400 kPa.