

POROUS CELL SUPPORT COMPRISING PLANT PROTEIN, AND CULTURED MEAT PREPARED USING SAME

TECHNICAL FIELD

The present disclosure relates to a porous scaffold including a plant protein and in-vitro meat produced by using the porous scaffold.

BACKGROUND ART

The world population is projected to reach 9 billion in 2050, and meat consumption by humans is also estimated to reach 4.65 million tons. In this regard, the Food and Agriculture Organization of the United Nations (FAO) has announced that additional production of 200 million tons of meat is necessary every year to meet the increasing demand for meat. Recently, 'in-vitro meat' is in the spotlight as a way to solve the problem of meat shortages. The term "in-vitro meat" refers to edible meat obtained by which cells are harvested from living animals and then proliferated by cell engineering technology, and is one of the cellular agriculture fields in which meat is obtained without going through a livestock rearing process.

By the development of in-vitro meat, there is a spreading awareness that some of various environmental pollution and ethical problems occurring in the process of raising and slaughtering livestock can be solved. The raising of livestock has been pointed out as a main contributor to greenhouse gas emissions. Thus, when the raising of livestock is reduced, effects such as expansion of farmland, reduction of soil erosion, and prevention of water pollution can be expected.

In addition, in-vitro meat has advantages in poor livestock environments and animal welfare related to slaughter, and can also exclude the risk of domestic animal infectious diseases, such as bovine spongiform encephalopathy and foot-and-mouth disease. Depending on a technological level, production of not only

beef, but also pork, chicken, and fish is possible in vitro. Therefore, large-scale production of in-vitro meat is expected to be an effective way to save resources and reduce environmental influences occurred by the livestock industry.

Meanwhile, regarding the production cost of in-vitro meat, \$ 325,000 was required to produce a single burger patty (100 g) at the beginning of the in-vitro meat development in 2013. Although the cost was reduced to \$1,986/100 g as of 2017, it was still very high. In addition, due to the limitations of the production technology, the currently produced in-vitro meat has been criticized for relatively inferior taste compared to real meat. In detail, in-vitro meat is not used as an ingredient in foods of which natural taste of meat is important as in steak, but is rather used as an ingredient for a burger patty. Thus, to eliminate the difference in taste with real meat, research is actively under way to make a real meat taste by mixing in-vitro meat with muscle, oil, bones, and the like. Furthermore, considering that a cell culture medium for the production of in-vitro meat requires horse or bovine fetal serum, there is a contradiction that as the production of in-vitro meat increases, livestock slaughter also increases.

Therefore, regarding the production method for in-vitro meat, technical research to solve high cost issues, enable mass production of in-vitro meat, and give a taste similar to real meat is required.

DESCRIPTION OF EMBODIMENTS

TECHNICAL PROBLEM

An objective of the present disclosure is to provide a porous scaffold including a plant protein composition.

Another objective of the present disclosure is to provide a method of producing the porous scaffold.

Another objective of the present disclosure is to provide a composition for producing in-vitro meat including the porous scaffold.

Another objective of the present disclosure is to provide a method of producing in-vitro meat by using the porous scaffold.

Another objective of the present disclosure is to provide in-vitro meat prepared by the above method.

SOLUTION TO PROBLEM

An aspect of the present disclosure provides a porous scaffold comprising a porous vegetable protein composition obtained by adding separated vegetable protein, porogen, and emulsifier to a solvent, followed by stirring and then adding a coagulant thereto.

The term "vegetable protein" refers to proteins found in plants, which may not contain all essential amino acids but are rich in antioxidant compounds such as flavonoids and anthocyanins. These antioxidants are known to have a significant impact not only on immune health but also on delaying the aging process. Additionally, vegetable proteins are known to be rich in dietary fibers such as beta-glucan and pectin, which provide abundant nutrients that are not adequately supplied by animal proteins.

The term "porogen" as used herein refers to any material available to produce a porous material, and is also referred to as a pore former or a pore-inducing agent. The porogen is edible, and may be one or more selected from the group consisting of agar, salt, calcium chloride, sodium carbonate, paraffin, polyethylene glycol, gelatin, and sucrose, but is not limited thereto. A material that is edible and can form a pore in the vegetable protein may be applicable without limitation.

The term "emulsifier" as used herein refers to a material that assists mixing of two materials that are difficult to mix with each other. The emulsifier is edible, and may be one or more selected from the group consisting of glycerin, propylene glycol, monoglyceride, diglyceride, lecithin, and soybean phospholipid, but not limited thereto. A material that is edible and can assist mixing of the

vegetable protein isolate with the porogen may serve as the emulsifier, and may be applicable without limitation.

The term "solvent" as used herein refers to a material that dissolves a solute. The solvent is edible, and may be one or more selected from the group consisting of glycerin, propylene glycol, monoglyceride, diglyceride, lecithin, and soybean phospholipid, but not limited thereto.

The emulsifier and the solvent may be the same materials. Any material may serve as the emulsifier and the solvent at the same time.

The coagulant is edible, and may be one or more selected from the group consisting of glucono-delta-lactone, calcium chloride, calcium sulfate, and magnesium chloride, but not limited thereto.

The porous scaffold may have a pore size in a range of about 10 μm to about 600 μm , 20 μm to about 550 μm , about 30 μm to about 500 μm , about 40 μm to about 450 μm , about 50 μm to about 400 μm , about 60 μm to about 350 μm , about 70 μm to about 300 μm , about 75 μm to about 250 μm , about 80 μm to about 200 μm , about 85 μm to about 150 μm , about 70 μm to about 130 μm , about 80 μm to about 120 μm , about 90 μm to about 110 μm , about 50 μm to about 600 μm , about 50 μm to about 400 μm , about 50 μm to about 300 μm , about 50 μm to about 250 μm , about 50 μm to about 200 μm , or about 50 μm to about 150 μm .

The porous scaffold may have elasticity in a range of about 1 kPa to about 10 kPa, about 2 kPa to about 8 kPa, about 3 kPa to about 8 kPa, about 1 kPa to about 5 kPa, about 4 kPa to about 10 kPa, about 6 kPa to about 10 kPa, about 1 kPa to about 3 kPa, or about 4 kPa to about 6 kPa.

The porosity of the porous cell scaffold may be 10 to 80%, 20 to 80%, 30 to 70%, 40 to 60%, 30 to 60%, 50 to 80%, or 60 to 80%. The porous scaffold may undergo a reversible phase transition to a solid state or a semi-solid state. In addition, the porous scaffold may be insoluble.

The plant protein may be isolated from at least one selected from the group consisting of nuts, beans, mushrooms, vegetables and grains.

In an embodiment, the porous scaffold may be produced by using an soy protein.

Cells may be dispensed in the porous scaffold.

Use of the porous scaffold may include three-dimensional cell culture.

In an embodiment, various types of cells may adhere, proliferate, or differentiate on the porous scaffold.

In an embodiment, adipose-derived stem cells, muscle cells, or adipocytes may be dispensed in the porous scaffold and cultured three-dimensionally.

The porous scaffold may have effects of not only stably and continuously culturing cells, but also exhibiting a culture pattern suitable for the cell properties. In addition, the porous scaffold according to an embodiment may have effects of enabling long-term culture even by the existing 2D culture methods of culturing cells in a cell culture dish or plate.

The cells dispersed in the porous scaffold may include cells selected from the group consisting of bacterial cells, yeast cells, mammalian cells, insect cells, and plant cells.

The cells may include, for example: eukaryotic cells from yeast, fungi, protozoa, plants, higher plants, and insects; cells from amphibians; or mammalian cells such as CHO, HeLa, HEK293, and COS-1. For example, the cells may include, as commonly used in the art, in vitro cells, graft cells, primary cell cultures (e.g., in vitro and ex vivo cell cultures), in vivo cells, and mammalian cells including human cells. In addition, an organism may be a yeast, a fungus, a protozoan, a plant, a higher plant, an insect, an amphibian, or a mammal.

The cells dispersed in the porous scaffold may include one selected from: cartilage cells; fibrocartilage cells; osteocytes; osteoblasts; osteoclasts; synovial cells; marrow cells; nerve cells; mesenchymal cells; epithelial cells; hepatocytes; muscle cells; stromal cells; vascular cells; stem cells; embryonic stem cells; mesenchymal stem cells; adipocytes; precursor cells derived from adipose tissues; peripheral blood precursor cells; stem cells isolated from adult tissues;

induced pluripotent stem cells (iPS cells), tumor cells derived from the cells above, and a combination thereof. In detail, the cells dispersed in the porous scaffold may be stem cells, adipocytes, precursor cells derived from adipose tissues, or muscle cells.

To induce differentiation or proliferation of the dispersed cells, the porous scaffold may further include a differentiation-inducing factor, a growth factor, a growth stimulator, a physiologically active material, or a cell-binding mediator such as a peptide or a polysaccharide.

In an embodiment, the growth factor or the growth stimulator may include: a cell adhesion mediator; a biologically active ligand; an integrin-binding sequence; a ligand; various growth and/or differentiation agents (e.g., an epithelial cell growth factor, IGF-I, IGF-II, TGF- β), a growth and differentiation factor; a stromal-derived factor (SDF-1); a vascular endothelial growth factor; a fibroblast growth factor; a platelet-derived growth factor; an insulin-derived growth factor; a transforming growth factor; a parathyroid hormone; a parathyroid hormone-related peptide, bFGF; a nerve growth factor (NGF) or a muscle morphogenic factor (MMF); a heparin-binding growth factor (HBGF), a transforming growth factor α or β , α fibroblast growth factor (FGF), epidermal growth factor (TGF- $\rightarrow$ EGF?), vascular endothelial growth factor (VEGF), SDF-1; TGF β superfamily factor; BMP-2; BMP-4; BMP-6; BMP-12; sonic hedgehog; GDF5; GDF6; GDF8; PDGF); small molecules affecting up-regulation of specific growth factors; tenascin-C; hyaluronic acid; chondroitin sulfate; fibronectin; decorin; thromboplastin; thrombin-derived peptides; TNF α/β ; matrix metalloproteinase (MMP); a heparin-binding domain; heparin; heparan sulfate; or DNA fragment or DNA plasmid thereof, short interfering RNA (siRNA), a trait infectious agent, or a mixture thereof.

Another aspect of the present disclosure provides a method of producing the porous scaffold, the method of producing a porous scaffold, the method comprising: adding a separated vegetable protein, porogen, and emulsifier into a

solvent, followed by stirring; and adding a coagulant thereto to obtain a porous vegetable protein composition.

The porogen, the emulsifier, the solvent, and the coagulant may respectively be the same as described above.

The method may further include, before the adding of the coagulant, steps of molding and freeze-drying the mixture. The molding may be performed in a casting mold having a desired shape and size. The freeze-drying may include: freezing at a temperature in a range of -40 °C to -240 °C, for 10 minutes to 3 hours and a drying step.

In detail, the freeze-drying may include: after coating the entire surface of a frozen plant protein composition with sodium bicarbonate, an adsorption step at room temperature. The sodium bicarbonate may serve to facilitate the flow of the coagulant to the inside of the plant protein composition by promoting the formation of pores on the surface of the composition. When the coagulant is added to the frozen plant protein composition without coating the sodium bicarbonate therewith, the coagulant may first act on the surface of the composition so that pores may be irregularly formed therein, thereby producing a structure unsuitable for culturing the cells.

In addition, the method of the present disclosure may further include washing the coagulated plant protein composition with distilled water or an organic solvent (for example, ethanol) and performing autoclave thereon, to remove impurities other than proteins.

Another aspect of the present disclosure provides a composition for producing in-vitro meat, comprising: the porous scaffold; and cells dispensed in the porous scaffold.

The term "in-vitro meat (also referred to as cultured meat, synthetic meat, cell-cultured meat, clean meat, or vat meat) refers to lean meat produced by cell-engineering techniques in which live animal cells are cultured to culture meat

without livestock farming. In-vitro meat is also referred to as artificial meat. Such in-vitro meat is not yet commercially produced, and several studies and projects are currently developing in-vitro meat techniques experimentally.

Cytodex 1[®] microcarrier previously known in the art to be used for the production of in-vitro meat is compared with the porous scaffold including the plant protein according to an embodiment, and the results of property comparison are shown in Table 1.

[Table 1]

	Cytodex 1 [®]	Embodiment of the present disclosure
Manufacturer	Cytiva (GE Healthcare)	DaNAgreen
Main ingredient	dextran	isolated soy protein
Main characteristics	(+)-surface charge	multiporous
Shape	spherical microcarrier	sponge-like sheet
Size	ø190 µm	25 x 25 x 2 mm
Max cell no. per gram	24 M	100 M
Scaffold no. per gram	172000	5
Max cell no. per scaffold	140	20 M
Cells attachment rate	~60%	100%
Price per 1kg meat	\$16,340	\$6.52
Price ratio	2506.1	1.0

The information on *46Cytodex 1[®] is obtained from the prior art document [Cytotechnology 2018 Apr; 70(2):503-512] and the website of the manufacturer (www.cytivalifesciences.com). The properties obtained in an embodiment are not limited to the entire disclosure, but are limited to the porous scaffold produced according to the embodiment. Another aspect of the present disclosure provides a method of producing in-vitro meat, the method including dispensing cells on the porous scaffold; culturing the cell three-dimensionally; and forming a three-dimensional cell aggregate.

The cells may include one or more selected from the group consisting of stem cells, muscle cells, and adipocytes, but are not limited thereto.

In an embodiment, in-vitro meat may be produced by dispensing muscle cells or adipocytes on the porous scaffold, and culturing the cells three-

dimensionally. In addition, since the porous scaffold consists of edible materials, in-vitro meat may be produced without any step of isolating the cultured cells from the porous scaffold.

In an embodiment, in-vitro meat may be produced by dispensing stem cells in the porous scaffold and culturing the cells to be differentiated into muscles or adipose tissues. In addition, since the porous scaffold consists of edible materials, in-vitro meat may be directly ingested without the need to separate the differentiated muscles or adipose tissues from the scaffold.

In an embodiment, in-vitro meat may be produced by the one-step process in which the cells and the porous scaffold are put into a bioreactor. When the cells and the porous scaffold are simultaneously put into a single incubator, the adhesion, proliferation, and differentiation of the cells occur at once, thereby constructing a mass production system.

Another aspect of the present disclosure provides in-vitro meat produced by the method disclosed herein, the in-vitro meat including the porous scaffold.

The porous scaffold and the in-vitro meat may respectively be the same as described above.

ADVANTAGEOUS EFFECTS OF DISCLOSURE

A scaffold including a plant protein according to an embodiment and in-vitro meat produced by using the scaffold consist of a plant protein, and thus cultured muscles or adipose tissues may be ingested together with the scaffold without being separated therefrom. By adjusting a ratio of muscle cells and adipocytes, in-vitro meat having desirable texture may be produced, and since various types of cells may adhere, proliferate, and differentiate on the scaffold, such a scaffold may be effectively utilized for mass production of in-vitro meat.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a photograph of a porous scaffold produced according to an embodiment, the porous scaffold including vegetable protein

FIG. 2 show the shapes of scaffolds that are previously known for cell culture, wherein FIG. 2a shows the shape of a concave microwell; FIG. 2b shows the shape of a porous scaffold; FIG. 2c shows the shape of a non-porous microbead; and FIG. 2d shows the shape of a porous microbead.

FIG. 3 shows the process(Fig.3a) and the result(Fig.3b) of measuring compressive strength of the porous scaffold including vegetable protein produced according to an embodiment.

FIG.4 shows the process (FIG. 4a) and the result (FIG. 4b) of measuring the tensile strength of the porous scaffold including vegetable protein produced according to an embodiment.

FIG.5 shows the process (FIG. 5a) and the result (FIG. 5b) of measuring the TPA(Texture Profile Analysis) of the porous scaffold including vegetable protein produced according to an embodiment.

FIG.6 is a photograph obtained by staining adipose-derived stem cells on the 9th day and the 15th day of cell culture after the cells are dispensed in the porous scaffold including vegetable protein produced according to an embodiment.

FIG. 7 is a schematic diagram illustrating a process of producing in-vitro meat in a bioreactor by using the porous scaffold including vegetable protein produced according to an embodiment.

FIG. 8 shows the state of culturing in-vitro meat, in-vitro chicken, and in-vitro pork by using the porous scaffold including vegetable protein produced according to an embodiment.

FIG. 9a is a photograph showing the results of live/dead assay performed on the 13th day and the 20th day in the process of culturing bovine muscle cells by using the porous scaffold produced according to an embodiment and Fig. 9b is a photograph showing the porous scaffold before and after the cultivation.

FIG. 10 is a photograph of the scaffold obtained by staining bovine muscle with Calcein-AM/Desmin in the process of culturing bovine muscle cells by using the porous scaffold produced according to an embodiment.

FIG. 11 is a photograph obtained by staining bovine muscle cells with Phalloidin/Desmin by using the porous scaffold produced according to an embodiment.

FIG. 12 is a photograph showing the results of live/dead assay performed on day 14 in the process of culturing chicken cardiomyocytes by using the porous scaffold produced according to an embodiment, and FIG. 12a shows the cells were first cultured by using the porous scaffold produced according to an embodiment in a 10 % DMEM culture medium for 7 days, and then, cultured for another 7 days after the culture medium was replaced with a 2 % DMEM culture medium and FIG. 12b shows the cells were cultured in a 10 % DMEM culture medium for 14 days and FIG. 12c shows the cells were cultured in a 2 % DMEM culture medium for 14 days.

FIG. 13 is the results of live/dead assay performed on the 13th day, the 17th day, and the 38th day in the process of culturing silki muscle cells by using the porous scaffold produced according to an embodiment.

FIG. 14 is the results of live/dead assay performed on the 14th day in the process of culturing bovine muscle cells and on the 8th day in the process of culturing chicken muscle cells, wherein the culturing was performed by using the porous scaffold produced according to an embodiment.

MODE OF DISCLOSURE

Hereinafter, preferable examples are provided to help understanding of the present disclosure. However, the following examples are only provided for easy understanding of the present disclosure, and the contents of the present disclosure are not limited by the following examples.

Example 1. Production of porous scaffold including vegetable protein

A porous scaffold including a vegetable protein was produced as follows.

10 g of powdered isolated soy protein (ISP) and 2 g of agar were added to 10 ml of edible glycerin (0409, ES food ingredient) and mixed in a steam-stirring machine at a speed of 60 rpm for 10 minutes (D-201, Daeduck Machinery). Then, 30 ml of sterilized tertiary distilled water was added and stirred at 5,000 rpm for 10 minutes using a high-speed stirrer (MaXtir™ 500S, DAIHAN-brand®), and transferred to a casting mold (100 mm X 100 mm X 2 mm) for molding into the desired shape, and frozen at -80 °C for 1 hour. Baking soda was sufficiently applied to all surfaces of the frozen sample, allowed for adsorption at room temperature for 4 hours, and dried at 60 °C for 4 hours. 2 g of gluconodelta-lactone (E575, JUNGBUNZLAUER S.A) was added to 1 L of sterilized tertiary distilled water, and the sample was coagulated at 80 °C for 2 hours. The resultant sample was washed five times each with 100% ethanol and sterilized tertiary distilled water at room temperature and then subjected to high-pressure sterilization twice while being immersed in 1 L of tertiary distilled water so as to remove non-protein impurities, thereby preparing a porous scaffold having a vegetable protein component (FIG. 1).

All materials for producing the porous scaffold are resources approved as food, and thus the porous scaffold can be ingested.

Example 2. Characterization of porous scaffold

Example 2.1 Morphological characterization of porous scaffold

The morphological properties of the porous scaffold of Example 1 were evaluated by using SKYSCAN1272 ex-vivo micro-CT (Bruker microCT, Belgium) under the following conditions.

- X-ray source: 40 kV, 200 uA, No-filter, rotation step 0.15°
- Resolution: 1 µm pixel resolution
- Staining with 0.1 % iodine-potassium iodide (IKI) overnight.

Section creation was analyzed by using a NRecon software, section rotation was analyzed by using a DataViewer software, analysis was performed by using a CTAn software, volume rendering creation was analyzed by using a CTVox software, and surface rendering was analyzed by using a CTAn+CTVol software, and results thereof are shown in Table 2.

[Table 2]

	Abbreviation	Measured value	Unit
Percent objective volume	Obj.V/TV	40.91257477	%
Structure thickness	St.Th	0.02461021	mm
Structure separation	St.Sp	0.09660629	mm
Open porosity	Po(op)	58.55148608	%
Total porosity	Po(tot)	59.08742523	%
Pore interconnectivity		58.55/59.09 = 99.1	%

- Percent object volume: the volume ratio occupied by a support.
- Structure thickness (structure thickness): the average spacing between pores.
- Structure separation: average size of pores.
- Open porosity: ratio of pores connected to the outside to total volume of a support.
- Total porosity: ratio of pores to total volume of a support.
- Pore interconnectivity: the ratio of pores connected to the outside out of all pores.

The pore interconnectivity of the cell scaffold prepared in Example 1 was about 99%. Accordingly, it was confirmed that the cell movement and the flow of the culture solution were smooth, making it suitable for the growth and differentiation of various cells.

Example 2.2 Physical characterization of porous scaffold

To analyze physical properties of the scaffold of Example 1, the compressive strength, the tensile strength, and Texture profile analysis(TSA) were measured by using a TXA™ texture analyzer (manufactured by Yeonjin S-

Tech Corp.)

Compressive strength was measured using the compression test method. The sample was placed on the plate and compressed into a square probe having the length and width of 10 mm, and then, measured at the initial strain rates of 0.1 [1/s] and 0.033 [1/s](FIG. 3A). Results thereof are as shown in Table 3 and FIG. 3B below.

[Table 3]

Testing Method	Compression Test			Temperature	21.6 degree Celsius	
Number of Lot				Humidity	Ambient	
Product Specification				Load Cell	30kgf	
	Width mm	Thickness mm	Length mm	Speed mm/s	Max Load gf	Max Stress kPa
0.1 Strain rate_1	7.765	7.260	1.778	0.178	724.368	126.009
0.033 Strain rate	7.726	7.660	1.689	0.051	637.388	105.756
Average	7.746	7.455	1.734	0.114	680.878	115.882
Standard deviation	0.028	0.276	0.063	0.090	61.504	14.320
Standard deviation(%)	0.4	3.7	3.6	78.7	9.0	12.4

The tensile strength was measured by clamping the sample to tensile grips of the texture analyzer and setting the initial strain rates to be 0.1[1/s] and 0.033[1/s] for measurement. Results thereof are as shown in Table 4 and FIG. 4B below.

[Table 4]

Testing Method	Tensile Test			Temperature	21.6 degree Celsius			
Number of Lot				Humidity	Ambient			
Product Specification				Load Cell	30 kgf			
	Width mm	Thickness mm	Length mm	Speed mm/s	Yield Load gf	Yield Stress kPa	Yield Elong. %	Max Load gf
Tensile Test 0.1 Strain rate	14.000	1.500	20.000	2.033	60.209	28.117	16.000	120.820
Tensile Test 0.033 Strain rate	14.000	1.500	20.000	0.617	52.489	24.511	14.297	116.659
Average	14.000	1.500	20.000	1.325	56.349	26.314	15.148	118.739
Standard deviation	0.000	0.000	0.000	1.001	5.459	2.549	1.204	2.942
Standard deviation(%)	0.0	0.0	0.0	75.5	9.7	9.7	7.9	2.5

Texture profile analysis (TPA) was measured using a texture analyzer, by placing the sample on a plate and compressing a cylinder probe with a width and a length of 25.4 mm and a diameter of 25.4mm by 50% compression twice at a rate of 60 mm/min (FIG. 5A). Results thereof are as shown in Table 5 and FIG.

5B below.

[Table 5]

	Square width Width	Square thickness Thickness	Sectional area SA	Gage length GL	Test speed SPEED	Hardness	Adhesiveness	Cohesiveness	Springiness	Gumminess	Chewiness	Resilience	Brittleness
	mm	mm	mm ²	mm	mm/min	kgf	kgf*sec	—	—	—	—	—	kgf
TPA_1	14.70	12.70	186.69	7.60	300.00	0.6729	0.0027	0.81	0.99	0.55	0.54	0.55	0.6729
TPA_2	14.20	13.70	194.54	7.64	300.00	0.6418	0.0013	0.82	1.02	0.53	0.54	0.55	0.6417
TPA_3	14.00	14.50	203.00	7.50	300.00	0.6922	0.0029	0.81	0.98	0.56	0.55	0.54	0.6921
Average	14.30	13.63	194.74	7.58	0.00	0.6689	0.0023	0.82	1.00	0.55	0.54	0.55	0.6689
Stand Dev.	0.36	0.90	8.16	0.07	0.00	1.6388	0.0057	2.00	2.44	1.34	1.33	1.34	1.6387

- Hardness: Force required to reach desired deformation.
- Cohesiveness: Force to sustain the shape of an objective as it is. In case of cohesiveness greater than adhesiveness, a sample does not stick to a probe.
- Springiness (also referred to as elasticity): Property in which a sample deformed by compression attempts to return to the original state after the force is removed.
- Gumminess: Force required to disintegrate a sample in a semi-solid state until it becomes a swallowable state that can be swallowed.
- Chewiness: Energy required to transfer a solid state of a sample into a swallowable state.
- Brittleness: Breaking or brittle property upon compression, which is represented as an intermediate peak that appears before reaching the maximum force of first compression, but may not appear depending on a sample.
- Resilience: Property of a sample to recover the original height thereof.

As such, it was confirmed that the scaffold produced according to Example 1 had suitable levels of compressive strength and tensile strength for the growth and differentiation of muscle cells, and for use as an edible scaffold used for the production of in-vitro meat, also had good texture with suitable elasticity and resilience properties and soft swallowability with suitable hardness and gumminess.

Example 2.3 Property comparison with existing scaffolds

The results of comparing the properties of the previously known scaffolds (e.g., concave microwell, porous scaffold, non-porous microbead, porous microbead, etc.) and the porous scaffold of Example 1 are shown in Table 6. For the properties of the previously known scaffolds, the prior art document [Materials

Science & Engineering C 103 (2019) 109782] was cited. The shapes of the previously known scaffolds are shown in FIG. 2.

[Table 6]

	Concave Microwell (a)	Porous Scaffold (b)	Non-porous microbead (c)	Porous	Example 1
Cell adhesion	N/A	***	***	***	*****
Media diffusion to the cells	*	***	*****	*****	*****
Endurance in bioreactors	*	*	*****	***	*****
Cell density when fully attached	N/A	*	***	*****	*****
Price	*****	*	***	*	*****
Material	Scaffold-free	Plastic	Plastic/Protein	Plastic/Protein	Protein
Suitability for in-vitro meat production	Research use products only	Research use products only	Not enough yield, aggregation occurs	Not strong enough to be used in bioreactors	Great for in-vitro meat application

As such, it was confirmed that, compared to the existing scaffolds for cell culture, the scaffold of Example 1 had high cell adhesion, excellent permeability of the culture medium, and high durability, and was made of materials suitable for the production of in-vitro meat.

Example 3. Three-dimensional culture of cells using porous scaffold

The porous scaffold of Example 1 having a diameter of 10 mm and a thickness of 2 mm was placed on a culture dish, and 10 % mesenchymal stem cell growth medium 2 (Promocell C-28009, C-39809) was added thereto. Then, 5.0×10^5 adipose-derived stem cells (ATCC PCS-500-011) were dispensed and cultured therein for 15 days for proliferation and histogenesis of the cells.

As a result, it was confirmed that the growth of cells continued as the culture period elapsed; the scaffold contracted as the cells adhered to the scaffold and grew thereon; and tissues were formed as the number of the cells increased and the void volume was minimized (FIG. 6).

Example 4. Production of in-vitro meat by using porous scaffold

In-vitro meat was produced by using the porous scaffold of Example 1. In

a bioreactor, cow muscle cells, chicken muscle cells, and pig muscle cells were directly put into the cell scaffold prepared in Example 1 and the cells were collectively cultured (FIG. 7) to prepare cultured beef, cultured chicken, and cultured pork (FIG. 8). As such, it was confirmed that, when the cells and the porous scaffold were simultaneously introduced into a single incubator, the one-step mass culture system, in which the adhesion, proliferation, and differentiation of the cells occur at once, can be established.

Example 4.1. Production of in-vitro beef by using porous scaffold

In a culture dish, the porous scaffold of Example 1 having a size of 25 mm X 25 mm X 2 mm, 1.7×10^6 Bovine #1 cells (i.e., primary skeletal muscle cells in DaNAgreen extracted from 150 g of rump muscles of a 36-month-old female Hanwoo (Korean native cattle, dressed weight: 339 kg, quality grade 2) slaughtered by Bunong Livestock Inc. on the same day as the experiment), and each of culture media (10 % DMEM of DMEM biowest L0103-500 and 2 % DMEM of FBS biowest S1480-500) were added, and the cells were cultured for 24 hours in a shaking incubator. As a result, it was confirmed that with the exception of 2%-3% residual cells, all cells were dispensed onto the porous scaffold.

In addition, during the 4 weeks of culture, the LIVE & DEAD assay was performed on day 13 and day 20, and few dead cells were observed within the scaffold, indicating that cell adhesion was well achieved, and the increase in live cells within a week confirmed that cell proliferation was well achieved on the scaffold (FIG. 9A). In addition, visual observation of the scaffold before and after culture showed that the scaffold after culture turned light pink, unlike the scaffold before culture, indicating that the proliferation of muscle cells occurred on the scaffold (FIG. 9B).

Subsequently, culture media (10 % DMEM of DMEM biowest L0103-500 and 2 % DMEM of FBS biowest S1480-500) were each added to the porous scaffold of Example 1 having a size of 25 mm X 25 mm X 2 mm. Then, 1.0×10^6 Bovine #2 cells (i.e., primary skeletal muscle cells extracted in DaNAgreen from

150 g of rump muscles of a 23-month-old female Hanwoo (Korean native cattle, dressed weight: 261 kg, quality grade 2) slaughtered by Samho Livestock on the same day as the experiment) were dispensed thereon and cultured for 7 days.

After the culture was completed, the cells were stained with Calcein-AM and Desmin Ab (FIG. 10) and Desmin Ab and Phalloidin (FIG. 11), and then observed. Calcein-AM was used to observe live cells and identify the morphology of differentiated cells, Desmin Ab, a muscle cell marker, was used to identify differentiated muscle cells, and Phalloidin was used to identify the morphology of differentiated cells. As a result, it was confirmed that differentiation into muscle cells was well made within the scaffold.

Accordingly, it was confirmed that in-vitro beef could be produced by culturing bovine muscle cells on the porous scaffold of Example 1.

Example 4.2. Production of in-vitro chicken by using porous scaffold

A culture media (10 % DMEM) were added to the porous scaffold of Example 1 having a size of 25 mm X 25 mm X 2 mm and, and 5.0×10^5 chicken Cardiomyocyte #1 (i.e., primary cardiomyocytes extracted from the heart by taking embryonic body from a fertilized egg of a general layer chicken at day 9 to day 11 of hatching in DaNAGreen) were dispensed thereon and cultured for 7 days to proliferate the cells. After 7 days, the culture medium was replaced with 2% DMEM, and the cells were cultured for another 7 days to differentiate into cardiomyocytes. On the 14th day after the culture, a live & dead assay was performed, which confirmed that overall the cells were growing well and producing culture meat (FIG. 12A).

Also, culture media (10 % DMEM and 2 % DMEM) were each added to the porous scaffold of Example 1 having a size of 25 mm X 25 mm X 2 mm. Then, 1.0×10^6 chicken Cardiomyocyte #1 (i.e., primary cardiomyocytes extracted from the heart by taking embryonic body from a fertilized egg of a general layer chicken on the 9th to 11th day of hatching by DaNAGreen) were dispensed on each culture

medium and cultured for 14 days. After the 14th day after the culture, a live & dead assay was performed, which confirmed that overall, the cells were growing well and producing culture meat (FIG. 12B and FIG. 12C).

Accordingly, it was confirmed that in-vitro chicken could be produced by culturing chicken muscle cells on the porous scaffold of Example 1.

Separately, in a culture dish, culture media (10 % DMEM of DMEM biowest L0103-500 and 2 % DMEM of FBS biowest S1480-500) were added to the porous scaffold of Example 1 having a size of 25 mm X 25 mm X 2 mm. Then, 1.0×10^7 Silkie #1 cells (i.e., primary skeletal muscle cells extracted from thigh by taking embryonic body from a fertilized egg of Silkie on the 9th to 11th day of hatching in DaNAgreen) were dispensed on each media and cultured for 5 weeks.

The live & dead assay was performed on days 13, 17, and 38 of culture and from the assay result, it was confirmed that the proliferation and differentiation of the Silkie muscle cells on the scaffold was well established (FIG. 13). In detail, live cells were full on the scaffold at the 2nd week of the culture, and the viability of the cells was confirmed even at the 5th week of the culture.

Example 4.3. Comparison of in-vitro beef and in-vitro chicken using porous scaffold

The culture media (10 % DMEM of DMEM biowest L0103-500 and 2 % DMEM of FBS biowest S1480-500) were added to the porous scaffold of Example 1 having a size of 25 mm X 25 mm X 2 mm. Then, 1.0×10^6 Bovine #1 cells and 2.0×10^6 Chicken #1 cells were each dispensed on each cell media and cultured for 4 weeks.

During culture, a live & dead assay was performed on day 14 for bovine and day 8 for chicken and results thereof were compared (FIG. 14). Chicken cells are smaller in size than bovine cells and have a faster doubling time, so it was confirmed that chicken cells filled the scaffold in a much shorter period of time when cultured for the same period. In addition, the cellular properties of both cultured meats were not inhibited by the scaffold, and it was confirmed that

different types of cultured meats have different types of muscle fibers, resulting in different flavors and tastes.

CLAIMS

1. A porous scaffold comprising a porous vegetable protein composition obtained by adding separated vegetable protein, porogen, and emulsifier to a solvent, followed by stirring and then adding a coagulant thereto.
2. The porous scaffold of claim 1, wherein the porogen includes at least one selected from the group consisting of agar, salt, calcium chloride, sodium carbonate, paraffin, polyethylene glycol, gelatin, and sucrose.
3. The porous scaffold of claim 1, wherein the emulsifier or the solvent includes at least one selected from the group consisting of glycerin, propylene glycol, monoglyceride, diglyceride, lecithin, and soybean phospholipid.
4. The porous scaffold of claim 1, wherein the coagulant includes at least one selected from the group consisting of glucono-delta-lactone, calcium chloride, calcium sulfate, and magnesium chloride.
5. The porous scaffold of claim 1, wherein the porous scaffold has a pore size in a range of 50 μm to 400 μm .
6. The porous scaffold of claim 1, wherein the separated vegetable protein is one that is separated from at least one selected from the group consisting of nuts, beans, mushrooms, vegetables, and grains.
7. The porous scaffold of claim 1, wherein cells are dispensed in the porous scaffold.
8. The porous scaffold of claim 1, wherein the porous scaffold is for three-dimensional cell culture.

9. A method of producing a porous scaffold, the method comprising:
adding a separated vegetable protein, porogen, and emulsifier into a solvent, followed by stirring; and
adding a coagulant thereto to obtain a porous vegetable protein composition.

10. A composition for producing in-vitro meat, comprising: the porous scaffold of claim 1; and cells dispensed in the porous scaffold.

11. A method of producing in-vitro meat, the method comprising dispensing a cell in the porous scaffold of claim 1 and three-dimensionally culturing the cell to form a three-dimensional cell aggregate.

12. The method of claim 11, wherein the cell is at least one selected from the group consisting of stem cells, muscle cells, and fat cells.

13. An in-vitro meat comprising a porous scaffold, the in-vitro meat produced by the method of claim 11.