

PROTEIN HYDROLYSATE COMPOSITION FROM CULTIVATED CELLS AND APPLICATIONS THEREOF

Cross Reference to Related Application

[0001] This application claims a provisional application, serial number 63/173,332, filed on April 9, 2021. This application further claims the priority of PCT application No. PCT/IB2020/060727 filed Nov. 14, 2020, which claims priority to a US provisional patent application serial number 62/942,568, filed on December 2, 2019. All applications identified above are hereby incorporated by reference in their entirety.

Technical Field

[0002] Embodiments discussed herein generally relate to cell hydrolysate composition from cultivated cells. Embodiments discussed herein also generally relate to the applications of the cell hydrolysate composition from cultivated cells and the process of making thereof. In particular, protein polypeptides and/or polypeptide fragments derived from cultivated cells.

Background

[0003] Animal meat is high in protein, and supplies all the amino acids needed to build the protein used to support body functions. Meat is often used to produce animal derived raw materials, including but not limited to, proteins, growth factors, cytokines, etc. Such animal derived raw materials have a lot of applications, which are often included in dietary supplements, hair care, skincare, cosmetic and wound care products, etc. The growth factors, cytokines, and extracellular matrix (ECM) proteins may stimulate skin tissue repair and regeneration. The hyaluronic acid derivatives may moisturize the skin and decrease wrinkles. The antioxidants may protect against aging-associated oxidative stress. The collagen may speed up healing. However, these animal derived raw materials are traditionally obtained from animals or fish that are reared on farms or caught in the wild. Similarly, plant derived raw materials (together with animal derived raw materials, "derived raw materials"), which may contain a lot of useful plant specific proteins, growth factors,

cytokines, etc, are now obtained from harvesting plants from farm or in the wild. However, creating derived raw materials from living animals and living plants has the following drawbacks.

[0004] First, it requires a constant supply of animal and plant sources. An increase in the demand for animal products causes more animals to suffer and be killed in farms and slaughterhouses. The growth in keeping livestock may also provide an additional burden to the environment or ecosystem. Raising livestock and farming could cause deforestation, increase in clean water consumption, increase in contamination to the environment (for example due to run off of animal wastes or pesticides and other chemicals used in farming to promote plant growth), excessive use of natural resources (for example, over farming and overfishing). These could lead to depletion of natural ecosystems and decrease biodiversity of the earth. Further, it may also give rise to animal abuse and welfare issues. In addition, instability in harvesting is the inherent characteristic of raising livestock and farming as it depends on a lot of uncontrollable factors including weather and climate.

[0005] Second, only targeted proteins are extracted and the rest of other functional proteins produced in the wild animals or wild plants are lost. Usually, the derived raw materials are created by extracting certain target/interested proteins from wild animals or wild plants. During extraction process, parts of animals and/or plants are harvested and the tissues therefrom (for example, animal skin and plant cell wall) are broken down through a series of processes. The processes may include the use of a combination of chemical, thermal and/or mechanical energy. The processes may lead to loss of certain proteins due to changes in pH, temperature and other conditions. As a result, non-target/non-interested proteins or nutrients are not captured in the extraction processes. However, it is desirable to also include those non-target/non-interested proteins in the derived raw materials because they involve trace amounts of functional proteins, which could be beneficial to certain purposes. For example, the total nutrition in oranges may be more beneficial to a human for a certain purpose compared to just vitamin C

extracted from the orange. Moreover, raising animals or growing plants only to obtain some usable parts to generate derived raw materials is highly inefficient.

[0006] Third, it involves the use of harmful chemicals. For example, extracting collagen from fish skin involves a series of steps including the steps of mixing the fish skins with alkaline and acidic solutions. This provides an additional burden to the environment or ecosystem. Also, it may contribute to potential occupational hazards. The derived raw materials may also contain harmful residual chemicals.

[0007] Fourth, there are safety concerns due to the presence of environmental contaminants (heavy metal, antibiotics, micro plastics, herbicides, fungicides, insecticides), adventitious agents (bacteria, viruses, fungi, transmissible spongiform encephalopathy agents), and allergens in the domesticated and wild animals and plants.

[0008] Fifth, there are difficulties in controlling the molecular profile and determining the consistency of animal-derived (e.g. animal serum) and plant-derived (e.g. plant extract, plant hydrolysate) raw materials. Each batch of animal-derived or plant-derived raw materials is created from different batches of animal and plant, which can deviate significantly due to variations of temperature, time of harvest, types of animal feed and fertilizers, presence of pest and parasites, etc. Some molecules in a certain batch may trigger an allergic reaction in some individuals.

[0009] Sixth, further, if the animal-derived and plant-derived raw materials are obtained from wild animals and wild plants, it is very difficult to trace back to the origin.

[0010] Alternatively, derived raw materials may be created by using recombinant organisms. However, this method involves the use of genetically modified organisms, which could be harmful to the environment when such organisms are released accidentally. Furthermore, only one (1) protein may be produced per production line. This method is inefficient for multiple proteins derived from raw materials. In addition, the protein produced by the recombinant organisms has to be further isolated and purified. The isolation and purification typically involve multiple steps which lead to an increase in production cost. Moreover, there may

be variations in the folding of the protein produced by this method. Some functions may be lost in some variations.

[0011] Alternatively, the spent or conditioned medium may also be a source of functional protein(s). "spent", "spent medium", "spent media", "conditioned media" or "conditioned medium" are the culture medium that has been incubated with cells. However, similar to the above methods, it has several drawbacks. Firstly, it requires a constant supply of animal and plant sources. The disadvantages thereof have been discussed in the foregoing and therefore not repeated here. Secondly, there are contamination concerns due to the presence of environmental contaminants (heavy metal, antibiotics, micro plastics, herbicides, fungicides, insecticides), adventitious agents (bacteria, viruses, fungi, transmissible spongiform encephalopathy agents), and allergens in the wild animals and wild plants sources. Thirdly, unwanted metabolites and wastes in the spent medium may affect the purity of the final product. Additional purification steps would lead to an increase in production costs.

[0012] In vitro meat production is the process by which muscle tissue or organ tissue from animals are grown in laboratories using cell culture techniques to manufacture meat and meat products. As used herein, in vitro meat and meat products includes animal protein products as well as non-meat products including soluble forms and solid forms in whole cell or hydrolyzed format. While still in an early stage of development, in vitro meat and meat products may offer a number of advantages over traditional meat products such as health and environmental advantages, and benefits to animal welfare. It is a next-generation and emerging technology that operates as part of a wider field of cellular agriculture, or the production of agricultural products from cell cultures.

[0013] Cells for the production of in vitro meat may be cells (e.g., muscle cells, somatic cells, stem cells, etc.) taken from animal biopsies, which may then be grown separately from the animal in culture media in a bioreactor or other type of sterile environment. The cells may grow into a semi-solid or solid form mimicking an animal organ by attaching to an edible three-dimensional scaffold that is placed in the bioreactor. Yet, the cells may also grow in suspension culture. The starter

cells may be primary cells directly obtained from the animal's tissues, or continuous cell lines. If grown under the right conditions in appropriate culture media, primary cells will grow and proliferate, but only a finite number of times that is related to the telomere length at the end of the cell's DNA. Continuous cell lines, on the other hand, can be cultured in vitro over an extended period. Cell biology research has established procedures on how to convert primary cells into immortal continuous cell lines. Primary cells may be transformed into continuous cell lines using viral oncogenes, chemical treatments, or overexpression of telomerase reverse transcriptase to prevent the telomeres from shortening.

Summary

- [0014]** While existing methods as mentioned in the background may fulfil certain requirements, for example, the recombinant method avoids animal scarification and the spent/conditioned medium method may provide a derived raw materials with more comprehensive trace components in relatively low cost compared to wild animals or wild plants harvesting method. However, none of the existing methods fulfill the growing demand of sustainability, low cost, whole cell components, complete cell protein/peptide portfolio and cruelty-free in one simple solution.
- [0015]** Of the many aspect of the invention, therefore, is comprising substantially all protein polypeptides and/or polypeptide fragments derived from substantially all the proteins in a cell from an in vitro cell culture; and (ii) free of wastes and metabolites from the culture media used in the in vitro cell culture.
- [0016]** It is an objective of the present invention to provide an alternative method of obtaining animal-derived raw materials and plant-derived raw materials using in vitro meat production and in vitro plant production respectively . The derived raw materials according to the present invention are free of environmental contaminants, adventitious agents and allergens. It also helps to limit animal suffering and sacrifice associated with the production of animal-derived materials.
- [0017]** Cell hydrolysate composition of the present invention may be applied as an active ingredient in dietary supplements, hair care, skincare, wound care, cosmetic or food products. Cell hydrolysate includes but is not limited to hyaluronic acid. The cell hydrolysate composition of the present invention comprises all protein

polypeptides and/or protein polypeptides fragments derived thereof from whole cell. In other words, the composition comprises multiple protein polypeptides and/or protein polypeptides fragments derived thereof instead of a single protein polypeptide. As such, the composition of the present invention is multifunctional. Given the foregoing, it is further an objective of the present invention to provide a cell hydrolysate composition through an efficient and environmentally friendly process. The protein hydrolysate composition of the present invention also provides improved batch-to-batch consistency and traceability for such composition. Some protein polypeptides can only be found in animal but not plants, vice versa. For example, collagen can only be found in animal cell but not plant cell. Generally, animal derived protein polypeptides have high efficacy to human than plant derived protein polypeptides do.

[0018] It is also an objective of the present invention to create further values for consumers or manufacturers in various industries/products, including but not limited to the dietary supplements, hair care, skincare, wound care, cosmetic, food products, supplements, drugs and other medicinal applications, as it simultaneously solves all drawbacks encountered by the conventional derived raw material creation method (i.e. extracting from wild animals/plants, recombinant DNA organisms and conditioned/spent medium). The present invention provides the following benefits:

- (1) no reliance on animal and/or plant sources;
- (2) no reliance on genetically modified organisms;
- (3) the capture of all functional proteins naturally produced by the subject cells;
- (4) Purity (no waste in the product);
- (5) No use of harmful chemicals;
- (6) simple downstream process, thereby lowering the cost; and
- (7) multi-functional instead of single-use/function/action.

[0019] The benefits of the present invention lead to the creation of new values that meet consumers' needs. It provides active ingredient of various products or the products itself (includes but not limited to dietary supplements, hair care, skincare,

wound care, cosmetic, food products, supplements, drugs and other medicinal applications) the following characteristics:

- (1) clean label due to the high purity and non-existence of waste or harmful chemicals in the derived raw materials;
- (2) sustainability due to little or low reliance on animal and/or plant sources;
- (3) non-chemical synthesis due to the use of biomaterials;
- (4) multi-functional due to its complete molecular profile with all functional proteins naturally produced by the subject cells; and
- (5) backed by scientific principles and test results.

[0020] According to some embodiments of the present invention, a cell hydrolysate composition, the composition comprising (i) a mixture of protein polypeptides and/or polypeptide fragments derived from collagen 1 α 1; (ii) a mixture of protein polypeptides and/or polypeptide fragments derived from collagen 1 β 1; (iii) a mixture of protein polypeptides and/or polypeptide fragments derived from connective tissue growth factor (CTFG); and (iv) a mixture of protein polypeptides and/or polypeptide fragments derived from Decorin.

[0021] Brief Description of the Drawings

[0022] The disclosure may be better understood by reference to the detailed description when considered in connection with the accompanying drawings. The components in the figures are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the disclosure.

[0023] FIG. 1 shows western blot analysis of proteins in a yellow croaker swim bladder tissue (labeled as A) and a yellow croaker swim bladder cell line (labeled as B).

[0024] FIG.2 is a flowchart of a method for generating cell hydrolysate from in vitro cell culture, according to some embodiments of the present disclosure.

[0025] FIG. 3A is a chart depicting gene expression upon exposure to the hydrolysate in regeneration circumstance. FIG. 3B is a chart depicting cell survival upon exposure to the hydrolysate in anti-oxidant circumstance. FIG. 3C is a chart depicting gene expression upon exposure to the hydrolysate in skin repair circumstance.

Detailed Description

[0026] Generating Cell Hydrolysate by In Vitro Cell Culture

[0027] The method of producing cell hydrolysate (i.e. animal-derived raw materials or plant-derived raw materials) from in vitro cell culture of the present invention offers a lot of benefits compared to those created from wild animals or plants.

[0028] First, since the cells are grown under contaminant-free and disease-free conditions, the culture medium does not contain environmental contaminants (heavy metal, antibiotics, micro plastics, herbicides, fungicides, insecticides) or adventitious agents (bacteria, viruses, fungi, transmissible spongiform encephalopathy agents). As a result, the animal-derived raw materials or plant-derived raw materials generated will be also contaminant-free and disease-free.

[0029] Second, an animal-component-free and chemically defined medium may be used to reduce the chance of triggering an allergic reaction of the user. This is achieved by substituting animal serum and animal-derived growth factors (e.g. bovine insulin) in the medium by recombinant growth factors. Plant extract/hydrolysate is not required in a chemically defined medium.

[0030] Third, the batch-to-batch consistency of the animal-derived raw materials or plant-derived raw materials may be significantly improved. This is because the nutritional profile (carbohydrates, amino acids, vitamins, minerals) of the basal medium is known and consistent and may be further refined using a chemically defined medium, i.e. a medium with known concentrations of all nutrients and growth factors.

[0031] Fourth, enhance traceability. Since the supply chain for every culture medium component is known, everything could be easily traced back to the origin.

[0032] Fifth, reduce animal suffering and sacrifice. Running the production process does not require a continuous supply of animal tissues from wild animals. Initially, the starter cells are purified from a small piece of animal tissue and developed into a cell line, which can be cryopreserved and propagated indefinitely in a culture medium. This limits animal suffering and sacrifice.

[0033] Sixth, reduce waste and enhance efficiency. Nutrients in the medium are directly supplied to cells for cell growth. Every cultivated cell is lysed to produce the cell hydrolysate. There is no waste of energy and nutrients for the growth of unused animal/plant parts, or life processes such as animal mating and locomotion.

[0034] Further, the present invention creates values for consumers or manufacturers of various industries/products, including but not limited to dietary supplements, hair care, skincare, wound care, cosmetic, food products, supplements, drugs and other medicinal applications, since it addresses all the drawbacks encountered in the conventional methods as mentioned in the background section. The present invention provides the following benefits: animal-derived raw materials and/or plant-derived raw materials from the products generated by in vitro meat production of the present invention offer a lot of benefits compared to those created from wild animals or plants.

[0035] First, it does not rely on a continuous feed of animal sources. For example, to produce more collagen-based cream via the conventional method, more animal body parts are needed which can result in more animal suffering or sacrifice. In the present invention, starter cells (not limited to stem cells, muscle cells, fibroblast cells, adipocytes) are purified from a small piece of animal tissue and established into a cell line. The cell line can be cryopreserved and stored in liquid nitrogen. When needed, the cell line can be thawed and propagated indefinitely under cell culture conditions to produce active ingredients (e.g. growth factors, ECM molecules). Therefore, the entire process is self-sustainable and causes little animal suffering/sacrifice.

[0036] Second, it does not rely on the animal source. In the present invention, starter cells (not limited to stem cells, muscle cells, fibroblast cells, adipocytes) are purified from a small piece of animal tissue and established into a cell line. The cell line is not genetically modified.

[0037] Third, the derived raw materials of the present invention are multi-functional because it includes a lot of trace amount of functional proteins in addition to the key target protein. Such a trace amount of functional proteins may be essential for

many cellular functions. A product created from derived raw materials with a functional protein profile closer to an extracted wild animal/plant part generally provides better performance than a product created from derived raw materials with a less complete functional protein profile. Even though functional proteins can be added into the derived raw materials through external replenishments, it is difficult to replenish a number of functional proteins. Also, the cost increases with the number of replenishments.

[0038] Fourth, the functional proteins created by the present invention do not contain waste since the ingredient in the growth medium is well controlled.

[0039] Fifth, the derived raw material created by the present invention does not contain harmful chemicals as the functional proteins are not extracted using harmful chemicals. The ingredient in the growth medium is well controlled.

[0040] Sixth, the functional proteins created by the present invention do not contain wastes and harmful chemicals, therefore, there is no need to further isolated and purified. Therefore, the production cost can be lower.

[0041] The benefits of the present invention allow the creation of new values that meet consumers' needs. It provides active ingredient of various product or the products itself (including but not limited to dietary supplements, hair care, skincare, wound care, cosmetic, food products, supplements, drugs and other medicinal applications) the following characteristics:

- (1) clean label due to the high purity and non-existence of waste or harmful chemicals in the derived raw materials;
- (2) sustainability due to little or low reliance on animal and/or plant sources;
- (3) non-chemical synthesis due to the use of biomaterials;
- (4) multi-functional due to its complete molecular profile with all functional proteins naturally produced by the subject cells; and
- (5) backed by scientific principles and test results.

[0042] As an example, FIG.1 shows western blot analysis of proteins in a yellow croaker swim bladder tissue (labeled as A) and a yellow croaker swim bladder cell line (labeled as B). It illustrates that in vitro cultured cells contain similar proteins

as the original tissue obtained from an animal. Therefore, it shows that the foregoing benefits can be fully realized by the present invention.

[0043] The method of producing cell hydrolysate composition from in vitro cell culture involves the steps of harvesting the cells from in vitro cell culture ("Harvesting Step"), lysing the harvested cells to release all the protein polypeptides from the harvested cells ("Lysing Step") and an optional digestion step the protein polypeptides from the lysing step are cut/cleaved ("Digestion Step"). In particular, the harvesting step may comprise the steps of separating the cells in the in vitro cell culture from the cell culture medium, which may further comprise the step of removing the cells from the in vitro cell culture container and/or the step of isolating the cells. The isolation may involve centrifugation to separate the cells from the cell culture medium and/or filtration to separate the cells from the cell culture medium. In some embodiments, membrane having pore size ranged from 5 μ m to 60 μ m may be used. The lysing step may further comprise lysing the cells by using mechanical means including but not limited to sonication, high pressure homogenizer, manual grinding and/or freeze/thaw cycles. Non-mechanical means may also be used to lyse the cells, including but not limited heating, osmotic shock, cavitation, alkali and/or detergent(s), acid hydrolysis and/or enzyme(s). For the optional digestion step, different enzyme(s) or chemical reagent(s) may be used, including but not limited to, subtilisin, chymotrypsin, Trypsin, carboxypeptidase, elastase, pepsin, proteinase K and/or cyanogen bromide.

[0044] The composition obtained from the foregoing method may be applied as a topical agent to dietary supplements, hair care, skincare, cosmetic, and wound care products. The hydrolysate may also be used as an active ingredient in various products, including but not limited to, dietary supplements, hair care, skincare, wound care, cosmetic, food products, supplements, drugs and other medicinal applications.

[0045] Referring now to the drawings, and with specific reference to FIG. 2, a method of producing cell hydrolysate from in vitro cell culture in one embodiment. In cell growing step 100, cells are first grown under controlled conditions using a

culture medium which is either partially defined (i.e. defined basal medium supplemented with FBS/plant hydrolysates/human platelet lysate) or a chemically defined medium (i.e. medium having defined concentrations of all nutrients and growth factors) that is devoid of animal or plant components. In yet some embodiments, at least one cell line may be used in the cell growing step 100. In some embodiments, the cell lines include stem cells, muscle cells, fibroblast cells, and adipocytes. In yet one specific embodiment, cultivated fish swim bladder cells are used in the cell growing step 100.

[0046] In detaching step 102, the cells are detached from its adhered surface and collect cell suspension into a tube.

[0047] In some embodiments, detaching step 102 may be skipped if the cells are collected from in vitro suspension cell culture.

[0048] The mix obtained from the detaching step 102 is then centrifuged ranged between 100 x g to 500 x g for 1-10 minutes in the centrifuging step 104, preferably 300 x g for 5 minutes. The supernatant is removed and cell pellet is obtained. In some embodiment, the cell pellet may be re-suspended in medium and perform the centrifuging step 104 more than once. In some embodiments, other speed and time may be used for the centrifugation.

[0049] In the resuspension step 106, the cell pellet is suspended in PBS at a predetermined volume. In some embodiments, the cell pellet is suspended in 1ml PBS. In some embodiments, the cell pellet can be suspended in buffer or saline other than PBS, for example, Hank's balanced salt solution.

[0050] In lysis step 108, the cells from step 106 are lysed by sonication.

[0051] Following cell lysis by sonication, the soluble fraction is isolated from the cell debris. Proteins in the soluble fraction are enzymatically digested into short functional peptides in digestion step 110. The desired protease of choice is added to the soluble fraction at a temperature ranged from 25 °C to 40 °C for 1-5 hours, preferably 30 °C for 2 hours. In some embodiments, the protease is pepsin, proteinase K or trypsin, preferably Proteinase K or Trypsin. Peptides having varies molecular size are obtained. In some embodiments, peptides having a molecular size ranged from 100 Daltons (Da) to 800 Da are favored. Preferably peptides

having a molecular size smaller than 500 Da is preferred because molecules having a molecular size greater than 500 Daltons (Da) do not effectively penetrate through the outermost epidermis and be absorbed by the underlying skin layers. In yet some other embodiments, peptides having a molecular size ranged from 100 Daltons (Da) to 500 Da is preferred. In some embodiments, an appropriate amount of protease is added to the cell suspension to break down cellular proteins into smaller peptides. Perform the digestion for 1-3 hours, preferably 2 hours, and keep the tube inside a temperature ranged from 25 °C to 40°C water bath, preferably 30 °C.

[0052] After the digestion step 110, the mix from digestion step 110 is then centrifuged ranged at 15000 x g to 25000 x g for 15-35 minutes in the isolation step 112 to clarify the liquid and removing any large debris, preferably 15000 x g for 20 minutes. In some embodiments, the isolation step 112 may be performed by filtering the mix from digestion step 110. In some embodiments, membrane having pore size ranged from 0.05µm to 0.5µm may be used. The supernatants from the micro centrifuge tubes are combined into a tube, preferably a 50 ml tube. Avoid disturbing the pellets in the micro centrifuge tubes.

[0053] In the termination step 114, the enzyme digestion activity is stopped by heating and/or dilution. In some embodiments, the termination step 114 may perform before the isolation step 112.

[0054] If the hydrolysate is not used immediately, in some embodiments, store it at a temperature ranged from +4 to -30 degrees Celsius, preferably -10 degree Celsius.

[0055] Cell Hydrolysate Compositions

[0056] A cell hydrolysate composition, the composition comprising substantially all protein polypeptides and/or polypeptide fragments derived from substantially all the proteins in a cell from an in vitro cell culture; and (ii) substantially free of wastes and metabolites from the culture media used in the in vitro cell culture comprising at least one of, including but not limited to, ammonia, lactate, pyruvate and putrescine. In some embodiments, the composition is substantially free of wastes and metabolites from the culture media used in the in vitro cell culture comprising

all of the ammonia, lactate, pyruvate and putrescine. The polypeptides and/or polypeptide fragments may range in size from about 100 Daltons (Da) to about 800 Da. In some embodiments, the average molecular size of the polypeptides and/or polypeptide fragments may be less than about 500 Da. In other embodiments, the average molecular size of the polypeptides and/or polypeptide fragments ranged from about 100 Da to about 500 Da. The cell hydrolysate compositions may be stored at a refrigerated temperature (i.e., +4° C to -30° C). In one embodiment, the cell hydrolysate composition may be stable for about one week to about four weeks. In another embodiment, the cell hydrolysate composition may be stable for about one month to about six months. In a further embodiment, the cell hydrolysate composition may be stable for more than about six months.

[0057] The cell hydrolysate composition may be dried. For example the cell hydrolysate composition may be freeze dried, vacuum dried or air dried. The temperature for drying is preferably less than 150° C.

[0058] In some embodiments, the protein polypeptides and/or polypeptide fragments are derived from at least one animal cell culture. In other embodiments, the protein polypeptides and/or polypeptide fragments are derived from at least one plant cell culture. In some embodiments, the protein polypeptides and/or polypeptide fragments are derived from a combination of animal cell culture and plant cell culture. In yet some embodiments, the protein polypeptides and/or polypeptide fragments are derived from yellow croaker swim bladder cell line. In some embodiments, the protein polypeptides and/or polypeptide fragments are derived from mutated cell cultures (both animals or plants). In some embodiments, the protein polypeptides and/or polypeptide fragments are derived from mutated or non-mutated human cell culture.

[0059] In some embodiments, the cell hydrolysate composition further comprises Lumican, Fibulin, Chondroitin, Chitosan, Glycosaminoglycan (chondroitin and heparan), Chondroadherin and Tropomyosin, etc.

[0060] In some embodiments, a cell hydrolysate composition comprises (i) a mixture of protein polypeptides and/or polypeptide fragments derived from

collagen 1 α 1; (ii) a mixture of protein polypeptides and/or polypeptide fragments derived from collagen 1 β 1; (iii) a mixture of protein polypeptides and/or polypeptide fragments derived from connective tissue growth factor (CTFG); and (iv) a mixture of protein polypeptides and/or polypeptide fragments derived from Decorin.

[0061] In yet some embodiments, the protein polypeptides and/or polypeptide fragments are derived from yellow croaker swim bladder cell line from an in vitro cell culture. In yet some embodiments, the protein polypeptides and/or polypeptide fragment are derived from yellow croaker swim bladder cell line from an in vitro cell culture and at least one animal cell line and/or plant cell line from an in vitro cell culture. In some embodiments, the protein polypeptides and/or polypeptide fragments are derived from mutated cell cultures (both animals or plants). In some embodiments, the protein polypeptides and/or polypeptide fragments are derived from mutated or non-mutated human cell culture.

[0062] In some embodiments, the composition is substantially free of wastes and metabolites comprising at least one of, but not limited to ammonia, lactate, pyruvate and putrescine. In some embodiments, the composition is substantially free of wastes and metabolites comprising all of ammonia, lactate, pyruvate and putrescine.

[0063] In some embodiments, the protein polypeptides and/or polypeptide fragments are derived from yellow croaker swim bladder cell line from an in vitro cell culture and enzymatically digested by Trypsin. In some embodiments, (i) the mixture of polypeptides and/or polypeptide fragments derived from collagen 1 α 1 comprises at least 1 polypeptide fragment selected from the group consisting of SEQ ID: 1- 112; (ii) a mixture of polypeptides and/or polypeptide fragments derived from collagen 1 β 1 comprises at least 1 polypeptide fragment selected from the group consisting of SEQ ID: 113 - 214; (iii) a mixture of polypeptides and/or polypeptide fragments derived from CTFG comprises at least 1 polypeptide fragment selected from the group consisting of SEQ ID: 215 – 249; and (iv) a mixture of polypeptides and/or polypeptide fragments derived from Decorin

comprises at least 1 polypeptide fragment selected from the group consisting of SEQ ID: 250 – 285.

[0064] In some embodiments, the protein polypeptides and/or polypeptide fragments are derived from yellow croaker swim bladder cell line from an in vitro cell culture and enzymatically digested by Trypsin. In some embodiments, (i) the mixture of polypeptides and/or polypeptide fragments derived from collagen 1 α 1 comprises at least 56 polypeptide fragments selected from the group consisting of SEQ ID: 1- 112; (ii) a mixture of polypeptides and/or polypeptide fragments derived from collagen 1 β 1 comprises at least 51 polypeptide fragments selected from the group consisting of SEQ ID: 113 - 214; (iii) a mixture of polypeptides and/or polypeptide fragments derived from CTFG comprises at least 17 polypeptide fragments selected from the group consisting of SEQ ID: 215 – 249; and (iv) a mixture of polypeptides and/or polypeptide fragments derived from Decorin comprises at least 18 polypeptide fragments selected from the group consisting of SEQ ID: 250 – 285.

[0065] In some embodiments, (i) the mixture of polypeptides and/or polypeptide fragments derived from collagen 1 α 1 comprises substantially all of the polypeptide fragments selected from the group consisting of SEQ ID: 1- 112; (ii) a mixture of polypeptides and/or polypeptide fragments derived from collagen 1 β 1 comprises substantially all of the polypeptide fragments selected from the group consisting of SEQ ID: 113 - 214; (iii) a mixture of polypeptides and/or polypeptide fragments derived from CTFG comprises substantially all of the polypeptide fragments selected from the group consisting of SEQ ID: 215 – 249; and (iv) a mixture of polypeptides and/or polypeptide fragments derived from Decorin substantially all of the polypeptide fragments selected from the group consisting of SEQ ID: 250 – 285.

[0066] In some embodiments, the protein polypeptides and/or polypeptide fragments are derived from yellow croaker swim bladder cell line from an in vitro cell culture and enzymatically digested by Proteinase K. In some embodiments, (i) the mixture of polypeptides and/or polypeptide fragments derived from collagen 1 α 1 comprises at least 1 polypeptide fragment selected from the group consisting

of SEQ ID: 286- 488; (ii) a mixture of polypeptides and/or polypeptide fragments derived from collagen 1 β 1 comprises at least 1 polypeptide fragment selected from the group consisting of SEQ ID: 489 - 657; (iii) a mixture of polypeptides and/or polypeptide fragments derived from CTFG comprises at least 1 polypeptide fragment selected from the group consisting of SEQ ID: 658 – 722; and (iv) a mixture of polypeptides and/or polypeptide fragments derived from Decorin comprises at least 1 polypeptide fragment selected from the group consisting of SEQ ID: 723 – 809.

[0067] In some embodiments, the protein polypeptides and/or polypeptide fragments are derived from yellow croaker swim bladder cell line from an in vitro cell culture and enzymatically digested by Proteinase K. In some embodiments, (i) the mixture of polypeptides and/or polypeptide fragments derived from collagen 1 α 1 comprises at least 101 polypeptide fragments selected from the group consisting of SEQ ID: 286- 488; (ii) a mixture of polypeptides and/or polypeptide fragments derived from collagen 1 β 1 comprises at least 84 polypeptide fragments selected from the group consisting of SEQ ID: 489 - 657; (iii) a mixture of polypeptides and/or polypeptide fragments derived from CTFG comprises at least 32 polypeptide fragments selected from the group consisting of SEQ ID: 658 – 722; and (iv) a mixture of polypeptides and/or polypeptide fragments derived from Decorin comprises at least 43 polypeptide fragments selected from the group consisting of SEQ ID: 723 – 809.

[0068] In some embodiments, (i) the mixture of polypeptides and/or polypeptide fragments derived from collagen 1 α 1 comprises substantially all of the polypeptide fragments selected from the group consisting of SEQ ID: 286-488; (ii) a mixture of polypeptides and/or polypeptide fragments derived from collagen 1 β 1 comprises substantially all of the polypeptide fragments selected from the group consisting of SEQ ID: 489 - 657; (iii) a mixture of polypeptides and/or polypeptide fragments derived from CTFG comprises substantially all of the polypeptide fragments selected from the group consisting of SEQ ID: 658 - 722; and (iv) a mixture of polypeptides and/or polypeptide fragments derived from Decorin substantially all

of the polypeptide fragments selected from the group consisting of SEQ ID: 723 - 809.

[0069] In some embodiments, the cell hydrolysate composition further comprises Lumican, Fibulin, Chondroitin, Chitosan, Glycosaminoglycan (chondroitin and heparan), Chondroadherin and Tropomyosin, etc.

[0070] Additionally, the invention also encompasses polypeptide fragments that are substantially similar in sequence to those selected from the group consisting of SEQ ID NOs: 1-809. In one embodiment, polypeptide fragment may have at least 80% sequence identity to a polypeptide fragment selected from the group consisting of SEQ ID NOs: 1-809. In another embodiment, the polypeptide fragment may have at least 90% sequence identity to a polypeptide fragment selected from the group consisting of SEQ ID NOs: 1-809.

[0071] It is also envisioned that the cell hydrolysate compositions of the invention may further comprise a non-hydrolyzed (i.e., intact) protein. The non-hydrolyzed protein may be present in an essentially intact preparation. Furthermore, the non-hydrolyzed protein may be isolated from a plant in vitro culture or isolated from an animal in vitro culture. The relative proportions of the protein hydrolysate and the non-hydrolyzed protein may vary depending on the application of the cell hydrolysate composition.

[0072] The multiple protein polypeptides, protein polypeptide fragments and/or other ingredients in the cell from the in vitro cell culture contained in the cell hydrolysate composition provide synergistic effects and benefits in various applications including but not limited to promoting general health, hair health, skin health, wound healing, joint health and collagen regulation and cartilage development.

[0073] In some embodiments, the cell hydrolysate composition has a pH ranged from 6.5-8.5. In yet some embodiments, the cell hydrolysate composition is water soluble. Yet in some embodiments, the color of the cell hydrolysate composition is ranged from colorless to pale yellow.

[0074] Products Comprising the Cell Hydrolysate Composition

[0075] The cell hydrolysate composition of the present invention may be applied as a topical agent to dietary supplements, hair care, skincare, cosmetic, and wound care products. The hydrolysate may also be used as an active ingredient in various products, including but not limited to, dietary supplements, hair care, skincare, wound care, cosmetic, food products, supplements, drugs and other medicinal applications.

[0076] Yet another aspect of the present invention, a pharmaceutical composition comprising the cell hydrolysate composition; and a pharmaceutical acceptable carrier.

[0077] For the dietary supplements, hair care, skincare, wound care, cosmetic or topical product, the hydrolysate from cultivated cells is rich in nutrients, contains multiple protein polypeptides and/or polypeptides fragments that stimulate skin cell repair and regeneration, and have a molecular size smaller than 500 Daltons (Da). The hydrolysate of the present invention can reach and take effect on the deep skin layers (dermis, hypodermis) as the hydrolysate of the present invention is small enough to pass through the stratum corneum and also maintains the key protein domains of growth factors and cytokines to elicit their functional activities.

[0078] Referring to FIG. 3A, the chart illustrates the difference in gene expression between skin cells being treated with cell hydrolysate composition and skin cells not being treated with cell hydrolysate in regeneration circumstance. It shows that the cell hydrolysate composition of the present invention boosts healthy protein metabolisms of the skin, increases collagen production, strengthens skin microstructure, revitalizes healthy complexion and skin tone from the inside out, and maintains skin and hair follicle integrity.

[0079] Referring to FIG. 3B, the chart illustrates the difference in cell survival between skin cells being treated with cell hydrolysate composition (Peptide A means cell hydrolysate composition of the present invention comprising SEQ ID: 286 – 809; and Peptide B means cell hydrolysate composition of the present invention comprising SEQ ID: 1- 285) and skin cells not being treated with cell hydrolysate in anti-oxidant circumstance (in this specific example, under hydrogen peroxide circumstance). It shows that the cell hydrolysate compositions of the

present invention, especially Peptide B, promotes the cell's own antioxidant defense, helps to protect the cells from harmful environmental irritants and pollutants, which cause premature skin aging, and increases skin cell survival from oxidative stress.

[0080] Referring to FIG. 3C, the chart illustrates the difference in gene expression between skin cells being treated with cell hydrolysate and skin cells not being treated with cell hydrolysate in skin repairing circumstance. It shows that the cell hydrolysate composition of the present invention strengthens skin barrier that protects the body from dehydration or trauma, repairs damage skin and prevents premature aging, and improves overall skin health for a youthful appearance.

[0081] The above description is illustrative and is not restrictive. Many variations of embodiments may become apparent to those skilled in the art upon review of the disclosure. The scope embodiments should, therefore, be determined not with reference to the above description, but instead should be determined with reference to the pending claims along with their full scope or equivalents.

[0082] One or more features from any embodiment may be combined with one or more features of any other embodiment without departing from the scope embodiments. A recitation of "a", "an" or "the" is intended to mean "one or more" unless specifically indicated to the contrary. Recitation of "and/or" is intended to represent the most inclusive sense of the term unless specifically indicated to the contrary.

[0083] While the present disclosure may be embodied in many different forms, the drawings and discussion are presented with the understanding that the present disclosure is an exemplification of the principles of one or more inventions and is not intended to limit anyone embodiment to the embodiments illustrated.

[0084] The disclosure, in its broader aspects, is therefore not limited to the specific details, representative system and methods, and illustrative examples shown and described above. Various modifications and variations may be made to the above specification without departing from the scope or spirit of the present disclosure, and it is intended that the present disclosure covers all such modifications and

variations provided they come within the scope of the following claims and their equivalents.

CLAIMS

What is claimed is:

1. A cell hydrolysate composition, the composition comprising substantially (i) all protein polypeptides and/or polypeptide fragments derived from substantially all the proteins in a cell from an in vitro cell culture; and (ii) free of wastes and metabolites from the culture media used in the in vitro cell culture.
2. The cell hydrolysate composition of claim 1, wherein the wastes and metabolites comprises at least one of ammonia, lactate, pyruvate and putrescine.
3. The cell hydrolysate composition of claim 1 is water soluble and the pH is ranged from about 6.5 to 8.5.
4. The cell hydrolysate composition of claim 1, wherein the polypeptides and/or polypeptide fragments have average molecular size ranged from about 100 Daltons (Da) to about 500 Da.
5. The cell hydrolysate composition of claim 1, wherein the in vitro cell culture is an animal cell culture or a plant cell culture.
6. The cell hydrolysate composition of claim 1, wherein the in vitro cell culture is yellow croaker swim bladder cells.
7. A cell hydrolysate composition, the composition comprising
 - (i) a mixture of protein polypeptides and/or polypeptide fragments derived from collagen 1 α 1;
 - (ii) a mixture of protein polypeptides and/or polypeptide fragments derived from collagen 1 β 1;
 - (iii) a mixture of protein polypeptides and/or polypeptide fragments derived from connective tissue growth factor (CTFG); and

(iv) a mixture of protein polypeptides and/or polypeptide fragments derived from Decorin.

8. The cell hydrolysate composition of claim 7, wherein the composition is substantially free of wastes and metabolites comprising at least one of ammonia, lactate, pyruvate and putrescine.

9. The cell hydrolysate composition of claim 7, wherein

(i) the mixture of polypeptides and/or polypeptide fragments derived from collagen 1 α 1 comprises at least 56 polypeptide fragments selected from the group consisting of SEQ ID: 1- 112;

(ii) a mixture of polypeptides and/or polypeptide fragments derived from collagen 1 β 1 comprises at least 51 polypeptide fragments selected from the group consisting of SEQ ID: 113 - 214;

(iii) a mixture of polypeptides and/or polypeptide fragments derived from CTFG comprises at least 17 polypeptide fragments selected from the group consisting of SEQ ID: 215 – 249; and

(iv) a mixture of polypeptides and/or polypeptide fragments derived from Decorin comprises at least 18 polypeptide fragments selected from the group consisting of SEQ ID: 250 – 285.

10. The cell hydrolysate composition of claim 7, wherein

(i) the mixture of polypeptides and/or polypeptide fragments derived from collagen 1 α 1 comprises at least 101 polypeptide fragments selected from the group consisting of SEQ ID: 286- 488;

(ii) a mixture of polypeptides and/or polypeptide fragments derived from collagen 1 β 1 comprises at least 84 polypeptide fragments selected from the group consisting of SEQ ID: 489 - 657;

(iii) a mixture of polypeptides and/or polypeptide fragments derived from CTFG comprises at least 32 polypeptide fragments selected from the group consisting of SEQ ID: 658 – 722; and

(iv) a mixture of polypeptides and/or polypeptide fragments derived from Decorin comprises at least 43 polypeptide fragments selected from the group consisting of SEQ ID: 723 – 809.

11. The cell hydrolysate composition of claim 9, wherein

(i) the mixture of polypeptides and/or polypeptide fragments derived from collagen 1 α 1 comprises at least 101 polypeptide fragments selected from the group consisting of SEQ ID: 286- 488;

(ii) a mixture of polypeptides and/or polypeptide fragments derived from collagen 1 β 1 comprises at least 84 polypeptide fragments selected from the group consisting of SEQ ID: 489 - 657;

(iii) a mixture of polypeptides and/or polypeptide fragments derived from CTFG comprises at least 32 polypeptide fragments selected from the group consisting of SEQ ID: 658 – 722; and

(iv) a mixture of polypeptides and/or polypeptide fragments derived from Decorin comprises at least 43 polypeptide fragments selected from the group consisting of SEQ ID: 723 – 809.

12. The cell hydrolysate composition of claim 9, wherein the cell hydrolysate is derived from an in vitro cell culture of a yellow croaker swim bladder cells.

13. The cell hydrolysate composition of claim 10, wherein the cell hydrolysate is derived from an in vitro cell culture of a yellow croaker swim bladder cells.

14. The cell hydrolysate composition of claim 11, wherein the cell hydrolysate is derived from an in vitro cell culture of a yellow croaker swim bladder cells.

15. A process of producing hydrolysate composition from in vitro cell culture comprising the steps of:

(i) harvesting the cells from in vitro cell culture;

(ii) lysing the harvested cells to release all the protein polypeptides from the harvested cells; and

(iii) optionally cutting or cleaving the protein polypeptides from the lysing step, wherein the harvesting step further comprises the step of isolating the cells from cell culture medium through centrifugation or filtration, wherein the lysing step further comprises at least one of the following: sonication, high pressure homogenizer, manual grinding, freeze/thaw cycles, heating, osmotic shock, cavitation, alkali and/or detergent(s), acid hydrolysis and/or enzyme(s); and wherein protein polypeptides from the lysing step are cut or cleaved by enzyme(s) or chemical reagent(s) comprising at least one of subtilisin, chymotrypsin, trypsin, carboxypeptidase, elastase, pepsin, proteinase K and/or cyanogen bromide.

16. The process of claim 15, wherein the step of cutting or cleaving the protein polypeptides is not optional.

17. The process of claim 16, wherein the protein polypeptides are cut or cleaved by proteinase K.

18. The process of claim 16, wherein the protein polypeptides are cut or cleaved by trypsin.

19. The process of claim 15, wherein the hydrolysate composition produced is the cell hydrolysate composition comprising substantially (i) all protein polypeptides and/or polypeptide fragments derived from substantially all the proteins in a cell from an in vitro cell culture; and (ii) free of wastes and metabolites from the culture media used in the in vitro cell culture.

20. The process of claim 15, wherein the hydrolysate composition produced is the cell hydrolysate composition comprising:

(i) a mixture of protein polypeptides and/or polypeptide fragments derived from collagen 1 $\alpha 1$;

(ii) a mixture of protein polypeptides and/or polypeptide fragments derived from collagen 1 β 1;

(iii) a mixture of protein polypeptides and/or polypeptide fragments derived from connective tissue growth factor (CTFG); and

(iv) a mixture of protein polypeptides and/or polypeptide fragments derived from Decorin.