

TOPICAL FORMULATIONS OF RECOMBINANT COLLAGENS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/827,662, filed April 1, 2019, which application is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Collagens and similar proteins are the most abundant proteins in the biosphere.

Collagens are structural proteins found in the skin, connective tissue, and bones of animals and other tissues. In humans, the amount of collagen present in the body is approximately one third of the total proteins and accounts for about three fourths of the dry weight of skin.

[0003] The structure of natural collagen can be a triple helix in which three polypeptide strands together form a helical coil. The individual polypeptide strands are composed of repeating triplet amino acid sequences designated as GLY-X-Y. X and Y can be any amino acid and the first amino acid is glycine. The amino acids proline and hydroxyproline are found in high concentrations in collagen. The most common triplet is glycine-proline-hydroxyproline (Gly-Pro-Hyp) accounting for approximately 10.5% of the triplets in collagen.

[0004] Gelatin is a product obtained by partial hydrolysis of certain (e.g., natural) collagen. Typically, gelatin is produced by acid hydrolysis, alkaline hydrolysis, and enzymatic hydrolysis or by exposing collagen to heat in an aqueous solution (e.g., boiling the bones and skins of animal, boiling fish scales, etc.).

[0005] Gelatin is used in many products including cosmetics, foods, pharmaceuticals, medical devices, photographic films, adhesives, binders, and many others. The physical and chemical properties of gelatin are tuned to the particular application. These physical/chemical properties include gel strength, melting point temperature, viscosity, color, turbidity, pH, isoelectric point, and others.

SUMMARY

[0006] In certain embodiments here are various polypeptides, compositions comprising such polypeptides, and methods of using such polypeptides and/or compositions thereof. In certain embodiments, such polypeptides comprise non-natural and/or recombinant polypeptides, such as comprising one or more amino acid sequence that is truncated relative to a natural collagen, such as a natural collagen described herein. In certain instances, such polypeptides are described herein as a “truncated collagen”. In specific embodiments, the polypeptide comprises one or more (e.g., two or more) truncated amino acid sequences of a natural human collagen. In other specific embodiments, the polypeptide comprises one or more (e.g., two or more) truncated

amino acid sequences of a natural jellyfish collagen. In one aspect, a method of providing a benefit to (e.g., increasing the firmness, elasticity, brightness, hydration, tactile texture, or visual texture of) skin (such as skin of an individual, such as a human) is provided. The method in some embodiments comprises topically applying a polypeptide described herein (e.g., a non-naturally occurring truncated collagen, such as described herein) or a formulation (e.g., that comprises a polypeptide, such as a non-naturally occurring truncated collagen) to the skin.

[0007] In a specific aspect, a method of decreasing lines or wrinkles present on skin or decreasing erythema of skin is provided. The method in some embodiments comprises topically applying a polypeptide described herein (e.g., a non-naturally occurring truncated collagen, such as described herein), or a formulation thereof, to the skin. In some embodiments, also provided herein is a formulation comprising a polypeptide described herein, such as a non-naturally occurring truncated collagen.

[0008] In certain instances, a polypeptide (e.g., a truncated collagen) described herein is useful in or for increasing the firmness, elasticity, brightness, hydration, tactile texture, and/or visual texture of skin. In some instances, a polypeptide (e.g., a truncated collagen) described herein is useful in or for decreasing lines or wrinkles present on skin or decreasing erythema of skin. In specific embodiments, the polypeptide (e.g., truncated collagen) is or comprises a truncated jellyfish collagen (e.g., a truncated amino acid sequence of a naturally occurring jellyfish collagen). In other specific embodiments, the polypeptide (e.g., truncated collagen) is or comprises a truncated human collagen (e.g., a truncated amino acid sequence of a naturally occurring human collagen).

[0009] In some embodiments, the polypeptide (e.g., truncated collagen) (e.g., useful in the methods disclosed herein) is or comprises a truncated amino acid sequence relative to a natural (e.g., human or jellyfish (Hydrozoan)) collagen. In some instances, such a polypeptide is referred to herein as a non-naturally occurring collagen. In specific embodiments, the non-naturally occurring collagen is or comprises an amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 27, and/or SEQ ID NO: 29, or a homolog thereof (e.g., having at least 85%, at least 90%, at least 95%, or at least 98% sequence identity thereto). In more specific embodiments, the non-naturally occurring collagen is an amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 27, and/or SEQ ID NO: 29, or a homolog thereof (e.g., having at least 85%, at least 90%, at least 95%, or at least 98% sequence identity thereto).

NO: 27, or SEQ ID NO: 29, or a homolog thereof (e.g., having at least 85%, at least 90%, at least 95%, or at least 98% sequence identity thereto).

[0010] In certain embodiments, compositions are provided herein. In some embodiments, such a composition comprises any polypeptides (e.g., truncated or non-natural collagen) described herein. In one aspect, provided are compositions that comprise any suitable amount, such as between 0.005% and 30% w/w, of any polypeptide (e.g., truncated or non-naturally occurring collagen) provided herein. The compositions can further comprise at least one additional ingredient comprising an excipient, a topical carrier, or a preservative.

[0011] In certain embodiments, a composition provided herein is a topical composition, such as a composition that is formulated and/or suitable for topical administration or use. In one aspect, provided herein is a method, such as of providing a benefit (e.g., as described herein) to the skin of an individual, the method comprising topically administering the topical composition to skin. In specific embodiments, the topical compositions are used in methods for decreasing skin damage, promoting the repair of damaged skin, or stimulating production of collagen by skin cells. In certain embodiments, the topical compositions are used in methods for increasing, promoting, stimulating, or otherwise increasing elastin production in the skin.

[0012] One aspect provides methods for applying the collagen or a composition comprising collagen to the skin of a subject.

[0013] As discussed herein, in some embodiments, a polypeptide provided herein is or comprises a truncated amino acid sequence relative to a natural (e.g., human or jellyfish (Hydrozoan)) collagen. In specific embodiments, such a polypeptide is a truncated collagen (e.g. comprises one or more truncated amino acid sequence relative to a natural collagen). In some embodiments, the truncated collagen is a jellyfish collagen or a human collagen. In various embodiments, the collagen is truncated at the C-terminal end, the N-terminal end, internally truncated, or truncated at both the C-terminal end and the N-terminal end (e.g., relative to a natural collagen). In one embodiment, the collagen is truncated at both the C-terminal end and the N-terminal end (e.g., relative to a natural collagen). In certain embodiments, a polypeptide provided herein is or comprises a truncated collagen of any suitable truncation, such as comprising a truncation at the C-terminal end, the N-terminal end, and/or one or more internal truncations. In some embodiments, truncation of the collagen is suitable to achieve beneficial results (e.g., an improved result relative to natural collagen and/or an additional benefit relative to natural collagen) and/or to shorten the length of the collagen while retaining one or more beneficial aspect of collagens. In some embodiments, polypeptides provided herein are or

comprise a collagen that is truncated in a manner such as to retain one or more topical benefit of collagen.

[0014] In some embodiments, a truncated collagen (amino acid sequence thereof) (e.g., of a polypeptide provided herein) is truncated at the C-terminal end by any suitable number of amino acid residues, such as up to 10, 10 to 800, 10 to 700, 10 to 500, 10 to 400, 10 to 300, 50 to 800, 50 to 700, 50 to 600, 50 to 500, 50 to 400, or the like. In certain embodiments, a truncated collagen (amino acid sequence thereof) (e.g., of a polypeptide provided herein) is truncated at the N-terminal end by any suitable number of amino acid residues, such as up to 10, 10 to 900, 10 to 800, 10 to 700, 10 to 500, 10 to 400, 10 to 300, 50 to 800, 50 to 700, 50 to 600, 50 to 500, 50 to 400, or the like. In some embodiments, a truncated collagen (amino acid sequence thereof) (e.g., of a polypeptide provided herein) is internally truncated by any suitable number of amino acid residues, such as up to 10, 10 to 900, 10 to 800, 10 to 700, 10 to 500, 10 to 400, 10 to 300, 50 to 800, 50 to 700, 50 to 600, 50 to 500, 50 to 400, or the like. In specific embodiments, a truncated collagen (amino acid sequence thereof) (e.g., of a polypeptide provided herein) is truncated at the C-terminal end by between 10 and 800 amino acids and/or truncated at the N-terminal end by between 10 and 800 amino acids. In another embodiment, the truncated collagen (amino acid sequence thereof) (e.g., of a polypeptide provided herein) is between 10 and 900 amino acids in length, between 10 and 800 amino acids in length, between 10 and 700 amino acids in length, between 10 and 600 amino acids in length, between 10 and 500 amino acids in length, between 10 and 400 amino acids in length, between 10 and 300 amino acids in length, between 10 and 200 amino acids in length, between 10 and 100 amino acids in length, between 10 and 50 amino acids in length, between 50 and 800 amino acids in length, between 50 and 700 amino acids in length, between 50 and 600 amino acids in length, between 50 and 500 amino acids in length, between 50 and 400 amino acids in length, between 50 and 300 amino acids in length, between 50 and 200 amino acids in length, or between 50 and 100 amino acids in length.

[0015] In certain embodiments, provided herein is a polypeptide that is or comprises an amino acid sequence of a human (e.g., human type 21) collagen. In specific embodiments, the truncated human collagen is a truncated human type 21 collagen. In various embodiments, truncation is according to any disclosure provided herein. In a specific embodiment, the truncated human type 21 collagen disclosed is SEQ ID NO: 16 (or a homolog thereof, such as having at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 98% sequence identity, or other sequence identity provided herein to an amino acid sequence of SEQ ID NO: 16). In various embodiments, such polypeptides are provided in any composition, formulation, or method provided herein.

[0016] In certain embodiments, provided herein is a polypeptide that is or comprises an amino acid sequence of a jellyfish (Hydrozoan) collagen. In various embodiments, the truncation is according to any disclosure provided herein. In a specific embodiment, the truncated jellyfish collagen disclosed is SEQ ID NO: 5 (or a homolog thereof, such as having at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 98% sequence identity, or other sequence identity provided herein to an amino acid sequence of SEQ ID NO: 5). In various embodiments, such polypeptides are provided in any composition, formulation, or method provided herein.

[0017] In certain embodiments, provided herein is a method comprising administering a polypeptide (e.g., that is or comprises a truncated collagen described herein) to the skin of an individual, such as to provide a benefit to the individual or to the skin thereof. In some instances, the benefit provided to the skin is improved firmness of the skin, improved elasticity of the skin, improved hydration of the skin, improved texture of the skin, improved brightness of the skin, decreased wrinkling of the skin, decreased erythema of the skin, improved collagen production in the skin, improved or increased elastin production in the skin, antioxidant protection to the skin, decreased redness of the skin, or other benefit, or combination of benefits, such as those described herein. In various instances, improvement in skin characteristics, or benefits provided by a method provided herein, is determined in any suitable manner, such as by use of instrumentation or by evaluation by a clinician. In one aspect, a method of increasing the firmness of skin is provided wherein the firmness of the skin is increased by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, or 75%. In one embodiment the firmness of the skin is measured using a cutometer.

[0018] Another aspect provides a method of increasing the elasticity of skin wherein the firmness of the skin is increased by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, or 75%. In one embodiment the elasticity of the skin is measured using a cutometer.

[0019] In another aspect, a method of increasing hydration of skin is provided wherein the hydration of the skin increases by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, or 75%. In one embodiment, skin hydration is measured on a corneometer.

[0020] In one aspect, a method of increasing the firmness of skin is provided wherein the firmness of the skin is increased. In one embodiment, the firmness of the skin is determined by an expert clinical grader.

[0021] In one aspect, a method of increasing the elasticity of skin is provided wherein the elasticity of the skin is increased. In one embodiment, the elasticity of the skin is determined by an expert clinical grader.

[0022] In one aspect, a method of increasing the brightness of skin is provided wherein the brightness of the skin is increased. In one embodiment, the brightness of the skin is determined by an expert clinical grader.

[0023] In another aspect, a method of increasing the tactile texture of skin is provided wherein the tactile texture of the skin is increased. In one embodiment, the tactile texture of the skin is determined by an expert clinical grader.

[0024] In one aspect, a method of increasing the visual texture of skin is provided wherein the visual texture of the skin is increased. In one embodiment, the visual texture of the skin is determined by an expert clinical grader.

[0025] In one aspect, a method of decreasing the lines or wrinkles present on skin is provided wherein the lines or wrinkles present on the skin is decreased. In one embodiment, the amount of lines or wrinkles present on of the skin is determined by an expert clinical grader.

[0026] In one aspect, a method of decreasing the erythema of skin is provided wherein the erythema of the skin is decreased. In one embodiment, the erythema of the skin is determined by an expert clinical grader.

[0027] Another aspect provides a method of stimulating collagen production in a skin cell. In one embodiment, the method comprises applying a non-naturally occurring truncated collagen or a formulation that comprises non-naturally occurring truncated collagen to the skin. In one embodiment, truncated jellyfish collagen or truncated human collagen stimulates collagen production. In a specific embodiment, the truncated human collagen is a truncated human type 21 collagen of SEQ ID NO: 16. In another specific embodiment, the truncated jellyfish collagen is a truncated jellyfish collagen of SEQ ID NO: 5.

[0028] Yet another aspect provides a method of stimulating collagen production in skin cells, wherein the collagen in skin increases by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, or at least 10%.

[0029] A topical formulation comprising a truncated collagen and one or more additional ingredient selected from the group consisting of water, oil, glycereth-8 esters, glycerin, coconut alkanes, hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, pentylene glycol, disodium EDTA, caprylyl glycol, chlorphenesin, and phenoxyethanol is disclosed. In one embodiment, the truncated collagen is a truncated jellyfish collagen or a truncated human collagen. In another embodiment, the truncated collagen is a truncated human type 21 collagen.

Yet another embodiment disclosed herein is a topical formulation comprising collagen and further comprising a vegetable oil. In one embodiment, the vegetable oil is olive oil.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0031] FIG. 1 illustrates an effect of an exemplary polypeptide provided herein (comprising a truncated human collagen amino acid sequence) on collagen type 1 protein secretion in fibroblasts.

[0032] FIG. 2A illustrates expression of collagen type 1 mRNA in fibroblasts treated with an exemplary polypeptide provided herein (comprising a truncated human collagen amino acid sequence).

[0033] FIG. 2B illustrates expression of elastin mRNA in fibroblasts treated with an exemplary polypeptide provided herein (comprising a truncated human collagen amino acid sequence).

[0034] FIG. 2C illustrates expression of fibronectin mRNA in fibroblasts treated with an exemplary polypeptide provided herein (comprising a truncated human collagen amino acid sequence).

[0035] FIG. 3 illustrates expression of IL-1 α in human primary keratinocytes treated with an exemplary polypeptide provided herein (comprising a truncated human collagen amino acid sequence).

[0036] FIG. 4 illustrates antioxidant capacity of an exemplary polypeptide provided herein (comprising a truncated human collagen amino acid sequence).

[0037] FIG. 5 illustrates viability of UVB-irradiated keratinocytes treated with an exemplary polypeptide provided herein (comprising a truncated human collagen amino acid sequence).

[0038] FIG. 6 illustrates skin elasticity after treatment with an exemplary polypeptide provided herein (comprising a truncated human collagen amino acid sequence).

[0039] FIG. 7 illustrates skin collagen content after treatment with an exemplary polypeptide provided herein (comprising a truncated human collagen amino acid sequence).

[0040] FIG. 8 illustrates quantification of skin redness after treatment with an exemplary polypeptide provided herein (comprising a truncated human collagen amino acid sequence).

[0041] FIG. 9 illustrates quantification of skin wrinkles after treatment with an exemplary polypeptide provided herein (comprising a truncated human collagen amino acid sequence).

[0042] **FIG. 10** illustrates collagen type 1 protein secretion by a human skin tissue model treated with an exemplary polypeptide provided herein (comprising a truncated jellyfish collagen amino acid sequence).

[0043] **FIG. 11** illustrates UVB-induced TT dimers in keratinocytes with treatment with an exemplary polypeptide provided herein (comprising a truncated jellyfish collagen amino acid sequence).

[0044] **FIG. 12** illustrates keratinocyte viability after UVB irradiation with treatment with an exemplary polypeptide provided herein (comprising a truncated jellyfish collagen amino acid sequence).

[0045] **FIG. 13** illustrates cell viability when treated with urban dust and an exemplary polypeptide provided herein (comprising a truncated jellyfish collagen amino acid sequence).

[0046] **FIG. 14** illustrates relative expression of IL-1 α induced by UVB after treatment with an exemplary polypeptide provided herein (comprising a truncated jellyfish collagen amino acid sequence).

[0047] **FIG. 15** illustrates antioxidant capacity of an exemplary polypeptide provided herein (comprising a truncated jellyfish collagen amino acid sequence).

[0048] **FIG. 16** illustrates skin hydration after treatment with an exemplary polypeptide provided herein (comprising a truncated jellyfish collagen amino acid sequence).

[0049] **FIG. 17** illustrates skin elasticity after treatment with an exemplary polypeptide provided herein (comprising a truncated jellyfish collagen amino acid sequence).

DESCRIPTION

[0050] In the following description, certain specific details are set forth in order to provide a thorough understanding of various embodiments of the disclosure. However, one skilled in the art will understand that the disclosure may be practiced without these details.

[0051] As used herein the term “about” generally refers to $\pm 10\%$.

[0052] The term “consisting of” means “including and limited to”. In general, a disclosure of “comprising” include a disclosure of “consisting of.”

[0053] The term “consisting essentially of” means that the composition, method, or structure may include additional ingredients, steps, and/or parts, but only if the additional ingredients, steps, and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method, or structure. In general, a disclosure of “comprising” includes a disclosure of “consisting essentially of.”

[0054] As used herein, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a compound” or “at least one compound” may include a plurality of compounds, including mixtures thereof.

[0055] Throughout this document, various embodiments of this disclosure may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0056] Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals there between.

[0057] The term “collagen” or “collagen-like” as used herein refers, in some instances, to a (e.g., monomeric) polypeptide that can associate with one or more collagen or collagen-like polypeptides to form a quaternary structure. Non-limiting examples of a collagen include a human type 21 alpha 1 collagen (e.g., SEQ ID NO: 31), a human type 1, alpha 2 collagen (e.g., SEQ ID NO: 32), and a jellyfish (Hydrozoan) collagen (e.g., SEQ ID NO: 33). In some instances, a collagen can be treated with acid, base, or heat to prepare a gelatin. The quaternary structure of natural collagen is a triple helix, typically composed of three polypeptides, but it should be noted that a “truncated collagen” or a polypeptide comprising a “truncated collagen” provided herein may or may not have such a quaternary structure, and does not necessarily have such a quaternary structure. In certain instances, of the three polypeptides that form natural collagen, two are usually identical and are designated as the alpha chain. The third polypeptide is designated as the beta chain. In certain instances, a typical natural collagen can be designated as AAB, wherein the collagen is composed of two alpha (“A”) strands and one beta (“B”) strand. In certain instances, polypeptides comprising “truncated collagens” provided herein may or may not have such structural elements. The term “collagen” or “collagen-like” may refer to the alpha chain polypeptide, the beta chain polypeptide, or both the alpha and beta chain polypeptides.

The term “procollagen” as used herein generally refers to polypeptides produced by cells that can be processed to naturally occurring collagen.

[0058] The term “expression vector” or “vector” as used herein generally refers to a nucleic acid assembly which is capable of directing the expression of the exogenous gene. The expression vector may include a promoter which is operably linked to the exogenous gene, restriction endonuclease sites, nucleic acids that encode one or more selection markers, and other nucleic acids useful in the practice of recombinant technologies.

[0059] The term “fibroblast” as used herein generally refers to a cell that synthesizes procollagen and other structural proteins. Fibroblasts are widely distributed in the body and found in skin, connective tissue and other tissues.

[0060] The term “fluorescent protein” generally refers to a protein that may be used in genetic engineering technologies used as a reporter of expression of an exogenous polynucleotide. The protein when exposed to ultraviolet or blue light fluoresces and emits a bright visible light. Proteins that emit green light include green fluorescent protein (GFP) and proteins that emit red light include red fluorescent protein (RFP).

[0061] The term “gelatin” as used herein generally refers to collagen that has been further processed by exposure to acid, base or heat. In some instances, gelatin solutions form reversible gels used in foods, cosmetics, pharmaceuticals, industrial products, medical products, laboratory culture growth media, and many other applications.

[0062] The term “gene” as used herein generally refers to a polynucleotide that encodes a specific protein, and which may refer to the coding region alone or may include regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence.

[0063] The term “histidine tag” generally refers to a 2-30 contiguous series of histidine residues on a recombinant polypeptide.

[0064] The term “host cell” generally refers to a cell that is engineered to express an introduced exogenous polynucleotide.

[0065] The term “keratinocyte” generally refers to a cell that produces keratins found in the epidermal layer of the skin.

[0066] The term “lactamase” as used herein generally refers to enzymes that hydrolyze antibiotics that contain a lactam (cyclic amide) moiety. “Beta-lactamase” or “ β -lactamase” are enzymes that hydrolyze antibiotics that contain a β -lactam moiety.

[0067] The term “non-naturally occurring” as used herein refers to a gene, polypeptide, or protein, for example, a collagen, that is not normally found in nature. The non-naturally

occurring collagens may be recombinantly prepared. The non-naturally occurring collagen may be a recombinant collagen. The non-naturally occurring collagen is, in one embodiment, a truncated collagen. Other non-naturally occurring collagen polypeptides include chimeric collagens. A chimeric collagen is a polypeptide wherein one portion of a collagen polypeptide is contiguous with a portion of a second collagen polypeptide. For example, a collagen molecule comprising a portion of a jellyfish collagen contiguous with a portion of a human collagen is a chimeric collagen. In another embodiment, the non-naturally occurring collagen comprises a fusion polypeptide that includes additional amino acids such as a secretion tag, histidine tag, green fluorescent protein, protease cleavage site, GEK repeats, GDK repeats, and/or beta-lactamase.

[0068] In general, disclosure of a collagen or truncated collagen provided herein, such as having a specific amino acid sequence, includes polypeptides having or comprising that precise amino acid sequence and homologs thereof. In some instances, homologs of an amino acid sequence provided herein may have a longer or shorter sequence and may have substitution of one or more amino acid residue of such amino acid sequence. Such homologs have a specific sequence identity to the recited sequence, such as in an amount provided herein. Sequence identity, such as for the purpose of assessing percent identity, may be measured by any suitable alignment algorithm, including but not limited to the Needleman-Wunsch algorithm (see, e.g., the EMBOSS Needle aligner available at www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html, optionally with default settings), the BLAST algorithm (see e.g. the BLAST alignment tool available at blast.ncbi.nlm.nih.gov/Blast.cgi, optionally with default settings), or the Smith-Waterman algorithm (see e.g. the EMBOSS Water aligner available at www.ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html, optionally with default settings). Optimal alignment may be assessed using any suitable parameters of a chosen algorithm, including default parameters. In some cases, a non-naturally occurring collagen may have at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% sequence identity to a sequence disclosed herein.

[0069] The term “protease cleavage site” generally refers to an amino acid sequence that is cleaved by a specific protease.

[0070] The term “secretion tag” or “signal peptide” generally refers to an amino acid sequence that recruits the host cell’s cellular machinery to transport an expressed protein to a particular location or cellular organelle of the host cell.

[0071] The term “truncated collagen” generally refers to a monomeric polypeptide that is smaller than a full-length collagen wherein one or more portions of the full-length collagen is not

present. Collagen polypeptides are truncated at the C-terminal end, the N-terminal end, truncated by removal of internal portion(s) of the full-length collagen polypeptide (e.g., an internal truncation), or truncated at both the C-terminal end and the N-terminal end. In a non-limiting embodiment, a truncated human collagen may comprise an amino acid sequence according to SEQ ID NO: 16, or a homolog thereof. In another non-limiting example, a truncated jellyfish collagen may comprise an amino acid sequence according to SEQ ID NO: 5, or a homolog thereof. Generally, a truncated collagen provided herein may have a function and/or provide a benefit (e.g., as provided herein) similar or substantially similar to that of a natural or a full-length collagen. In some cases, a truncated collagen provided herein may have improved or increased function and/or benefit (e.g., as provided herein) as compared to a natural or a full-length collagen.

[0072] When used in reference to an amino acid position, a “truncation” is inclusive of said amino acid position. For example, an N-terminal truncation at amino acid position 100 of a full-length protein means a truncation of 100 amino acids from the N-terminus of the full-length protein (i.e., the truncated protein is missing amino acid positions 1 through 100 of the full-length protein). Similarly, a C-terminal truncation at amino acid position 901 of a full-length protein (assuming a 1000 amino acid full-length protein) means a truncation of 100 amino acids from the C-terminus (i.e., the truncated protein is missing amino acid positions 901 through 1000 of the full-length protein). Similarly, an internal truncation at amino acid positions 101 and 200 means a internal truncation of 100 amino acids of the full-length protein (i.e., the truncated protein is missing amino acid positions 101 to 200 of the full-length protein).

[0073] In some embodiments, the cell culture may further comprise one or more of: ammonium chloride, ammonium sulfate, calcium chloride, amino acids, iron(II) sulfate, magnesium sulfate, peptone, potassium phosphate, sodium chloride, sodium phosphate, and yeast extract.

[0074] The host bacterial cell may be cultured continuously or discontinuously; in a batch process, a fed-batch process or a repeated fed-batch process.

[0075] In general, the signal sequence may be a component of the expression vector, or it may be a part of the exogenous gene that is inserted into the vector. The signal sequence selected may be one that is recognized and processed (e.g., cleaved by a signal peptidase) by the host cell. For bacterial host cells that do not recognize and process the native signal sequence of the exogenous gene, the signal sequence may be substituted by any commonly known bacterial signal sequence. In some embodiments, recombinantly produced polypeptides can be targeted to the periplasmic space using the DsbA signal sequence. Dinh and Bernhardt, J Bacteriol, Sept. 2011, 4984-4987.

[0076] In one aspect, a non-naturally occurring collagen that is produced by a host cell is provided. The non-naturally occurring collagen can be a jellyfish collagen or human collagen. The non-naturally occurring collagen may be a truncated collagen. The truncation may be an internal truncation (e.g., a truncation of an internal portion), a truncation at the N-terminal portion of the collagen, a truncation at the C-terminal portion of the collagen, or a truncation at both the C-terminal end and the N-terminal end. The collagen may be truncated by a truncation of between 50 amino acids and 1000 amino acids, between, 50 amino acids and 950 amino acids, between 50 amino acids and 900 amino acids, between 50 amino acids and 850 amino acids, between 50 amino acids and 800 amino acids, between 50 amino acids and 850 amino acids, between 50 amino acids and 800 amino acids, between 50 amino acids and 750 amino acids, between 50 amino acids and 700 amino acids, between 50 amino acids and 650 amino acids, between 50 amino acids and 600 amino acids, between 50 amino acids and 650 amino acids, between 50 amino acids and 500 amino acids, between 50 amino acids and 450 amino acids, between 50 amino acids and 400 amino acids, between 50 amino acids and 350 amino acids, between 50 amino acids and 300 amino acids, between 50 amino acids and 250 amino acids, between 50 amino acids and 200 amino acids, between 50 amino acids and 150 amino acids, or between 50 amino acids and 100 amino acids. In another embodiment, the collagen may be truncated by about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 650, 700, 750, 800, 850, 900, 950, or 1000 amino acids. The non-naturally occurring collagen may be encoded by a portion of a polynucleotide sequence or the entire polynucleotide sequence disclosed herein.

[0077] A truncated collagen disclosed herein may comprise a truncation relative to a full-length collagen. In some embodiments, a truncated collagen disclosed herein may comprise a truncation relative to a full-length human type 21 alpha 1 collagen. In some embodiments, a truncated collagen disclosed herein may comprise a truncation relative to a full-length human type 1 alpha 2 collagen. In some embodiments, a truncated collagen disclosed herein comprise a truncation relative to a full-length jellyfish (Hydrozoan) collagen. Non-limiting examples of full-length collagens are provided in Table 1 below.

Table 1. Full-length collagen amino acid sequences

Collagen	Amino Acid Sequence
Human type 21 alpha 1 collagen	MAHYITFLCMVLVLLQLNSVLAEDGEVRSSCRTAPTDLVFILDGYSYVGPFENFEIVKKWL VNITKNFDIGPKFIQVGVVQYSYDYPVLEIPLGSYDSGEHLTAAVESILYLGNTKTGKAI QFALDYLFKSSRFLTKIAVVLTDGKSQDDVKDAAQAARDSKITLFAIGVGSETEDAELR AIANKPSSTYVFYVEDYIAISKIREVMKQKLCEESVCPTRIPVAARDERGFIDILLGLDVN

	KKVKKRIQLSPKKIKGYEVTSKVDLSELTSNVFPEGLPPSYVVFSTQRFKVKKIWDLWRI LTIDGRPQIAVTLNGVDKILLFTTTSVINGSQVVTFANPQVKTLFDEGWHQIRLLVTEQD VTLYIDDQQIENKPLHPVLGILINGQTQIGKYSKKEETVQFDVQKLRIYCDPEQNNRETA CEIPGFNGECLNGPSDVGSTPAPCICPPGKPGGLQGPKGDPGLPGNPGYPGQPGQDGKPGY QGIAGTPGVPGSPGIQGARGLPGYKGEPRDGDGDRGLPGFPLHGMFGSKGEMGAKGD KGS PGFYGKKGAKGEKGNAGFPGLPGPAGEPGRHGKDGMLGSPGFKGEAGSPGAPGQDGT RGEPIPGFPGNRGLMGQKGEIGPPGQQGGKKGAPGMPGLMGSNGSPGPGTTPGSKGSKGE PGIQGMPGASGLKGEPEGATGSPGEPGYMGLPGIQGKKGDKGNQGEKGIQGGQKGENGRQGI PGQQGIQGGHGAKEGERGEKGEPEGVRGAIGSKGESGVDGLMGPAGPKGQPGDPPGQPPGL DGKPGREFSEQFIRQVCTDVIRAQLPVLLQSGRI RNC DHCLS QHGSPIGPFGPIGPEG PRGLPGLPRDGVPLVGVPRPGVRGLKGLPRNGEKSGQGFYGPGEQPGPPGPEGP PGISKEGPPGDPGLPGKDGHDGKPGIQGQPGPPGICDPSLCFSVIARRDFFRKGPNY (SEQ ID NO: 31)
Human type 1 alpha 2 collagen	MLSFVDTRTLLLLAVTLCLATCQSLQEETVRKGPAGDRGRGERGPPGPPGRDGEDGPTG PPGPPGPPGPPGLGNGFAAQYDGKGVGLPGPMGLMGRGPPGAAGAPGPGQFGPAGEP GEPGQTGPAGARGPAGPPGKAGEDGHPGKPRPGERGVVGPQGARGFPPTPLPGFKGIR GHNGLDGLKQGPAGPVKGEFGAPGENGTGQGTGARGLPGERGRVGAEPGARGSDGVS GPVGPAGPIGSAGPPGFPAGPGKGEIGAVGNAGPAGPAGPRGEVGLPGLSGPVGPPGNP GANGLTGAKGAAGLPVAGAPGLPGPRGIPGPVGAAGATGARGLVGEPGAGSKGESGNK GEPGSAGPQGGPPGPGSGEEGRGPNGEAGSAGPPGPPGLRSGPSRGLPGADGRAGVMGPP GSRGASGPAGVRGPNAGRPGEPLMGPRGLPGSPGNIGPAGKEGPVGLPGIDGRPGPI GPAGARGEPPNIGFPGPKGPTGDPGKNGDKGHAGLAGARGAPGPDGNGAQQGPPGPQGVQ GKGEGQGGPPGPGQGLPGPSGPAGEVVGKGERGLHGEFGLPGPAGPRGERGPPGESGAA GPTGPIGSRGPGSPGPDGNKGEPPVVGAVGTAGPSGPGSLPGERGAAGIPGGKGEKGE GLRGEIGNPGRDGARGAPGAVGAPGAGATGDRGEAGAAGPAGPAGPRGSPGERGEVGA GPNGFAGPAGAAGQPGAKGERGAKGPKGENGVVGPTGPVGAAGPAGNPPGPPAGSRGDG GPPGMTGFPAGAAGRTGPPGPGSGISGPPGPPGPGAGKEGLRGPGRDQGPVGRTEVGA GFAGEKGPSGEAGTAGPPGTGPGQGLLAPGILGLPGSRGERGLPGVAGAVGEPGLGIA GPPGARGPPGAVGSPGVNAPGEAGRDGNPNDGPPGRDQPGHKGERGYPGNI GPVGA GAPGPHGPVGPAGKHGNRGETGPSGPVGPAGAVGPRGSPGQIRGDKGEPEKGPRLP GLKGHNLQGLPGIAGHHGDQAGPSVGPAGPRGAPGSGPAGKDGRTGHPGTGVPATIR GPQGHQGPAGPPGPPGPPGPGVSGGGYDFGYDGDYRADQPRAPSLRPKDYEVDA TLK SLNNQIETLLTPEGSRKNPARTCRDLRLSHPEWSSGYWIDPNQGCMTDAIKVYCDFSTG ETCIRAQPENIPAKNWYRSSKDKKHVWLGETINAGSQFEYNVEGVTSKEMATQLAFMRL ANYASQNITYHCKNSIAYMDEETGNLKKAVILQGSNDVELVAEGNSRFTYTVLVDGCSKK TNEWGKTII EYKTNKPSRLPFLDIAPLDIGGADQEFFVDIGPVCFK (SEQ ID NO: 32)
Jellyfish (Hydrozoan) collagen	GPQGVVGADGKDGTPEKGEQGRGAAGKQSGADGARGPLGSIGQQGARGEPGDPGSP GLRGDTGLAGVKGVAGPSGRPGQPGANGLPGVNGRGLRGKPGAKGIAGSDGEAGESGAP GQSGPTGPRGQRPSPGEDGNPGLQGLPGSDGEPGEEGQPRSGQPGQGGPRGSPGEVGP GSKGPSGDRGDRGERGVPGQTGSAGNVGEDGEQGGKGVGDGASGPSALGARGPPGSRGDT GAVGPPGPTGRSGLPGNAGQKGPSGEPGSPGKAGSAGEQGP PGKDGSGNPGSPGKEGER GLAGPPGPDGRRGETGSPGIAGALGKPGLEGPKYPGLRGRDGTNGKRGEQGETGPDGVR GIPGNDGQSGKPGIDGIDGTNGQPEAGYQGGRGTRGQLGETGDVGQNGDRGAPGPDGSK GSAGRPLR (SEQ ID NO: 33)

[0078] In some cases, a truncated collagen as described herein may comprise an N-terminal truncation at any amino acid position between amino acid positions 1 and 548; between amino acid positions 1 and 553; between amino acid positions 1 and 558; between amino acid positions 1 and 563; between amino acid positions 1 and 568; or between amino acid positions 1 and 573 of SEQ ID NO: 31. In some cases, a truncated collagen as described herein may comprise a C-terminal truncation at any amino acid position between amino acid positions 726 and 957; between amino acid positions 731 and 957; between amino acid positions 736 and 957; between amino acid positions 741 and 957; between amino acid positions 746 and 957; between amino

acid positions 751 and 957; or between amino acid positions 756 and 957 of SEQ ID NO: 31. In some cases, a truncated collagen as described herein may comprise both an N-terminal truncation and a C-terminal truncation. For example, a truncated collagen as described herein may comprise an N-terminal truncation at any amino acid position between amino acid positions 1 and 548; between amino acid positions 1 and 553; between amino acid positions 1 and 558; between amino acid positions 1 and 563; between amino acid positions 1 and 568; or between amino acid positions 1 and 573 of SEQ ID NO: 31; and a C-terminal truncation at any amino acid position between amino acid positions 726 and 957; between amino acid positions 731 and 957; between amino acid positions 736 and 957; between amino acid positions 741 and 957; between amino acid positions 746 and 957; between amino acid positions 751 and 957; or between amino acid positions 756 and 957. In a specific embodiment, a truncated collagen disclosed herein may comprise an N-terminal truncation at amino acid position 558 of SEQ ID NO: 31; and a C-terminal truncation at amino acid position 746 of SEQ ID NO: 31.

[0079] In some cases, a truncated collagen as described herein may comprise an N-terminal truncation at any amino acid position between amino acid positions 1 and 401; between amino acid positions 1 and 406; between amino acid positions 1 and 411; between amino acid positions 1 and 416; between amino acid positions 1 and 421; between amino acid positions 1 and 426; or between amino acid positions 1 and 431 of SEQ ID NO: 32. In some cases, a truncated collagen as described herein may comprise a C-terminal truncation at any amino acid position between amino acid positions 585 and 1366; between amino acid positions 590 and 1366; between amino acid positions 595 and 1366; between amino acid positions 600 and 1366; between amino acid positions 605 and 1366; between amino acid positions 610 and 1366; between amino acid positions 615 and 1366; or between amino acid positions 620 and 1366 of SEQ ID NO: 32. In some cases, a truncated collagen as described herein may comprise both an N-terminal truncation and a C-terminal truncation. For example, a truncated collagen as described herein may comprise an N-terminal truncation at any amino acid position between amino acid positions 1 and 401; between amino acid positions 1 and 406; between amino acid positions 1 and 411; between amino acid positions 1 and 416; between amino acid positions 1 and 421; between amino acid positions 1 and 426; or between amino acid positions 1 and 431 of SEQ ID NO: 32; and a C-terminal truncation at any amino acid position between amino acid positions 585 and 1366; between amino acid positions 590 and 1366; between amino acid positions 595 and 1366; between amino acid positions 600 and 1366; between amino acid positions 605 and 1366; between amino acid positions 610 and 1366; between amino acid positions 615 and 1366; or between amino acid positions 620 and 1366 of SEQ ID NO: 32. In a specific embodiment, a

truncated collagen as provided herein may comprise an N-terminal truncation at amino acid position 416 of SEQ ID NO: 32; and a C-terminal truncation at amino acid position 605 of SEQ ID NO: 32.

[0080] In some cases, a truncated collagen as described herein may comprise an N-terminal truncation at any amino acid position between amino acid positions 1 and 101; between amino acid positions 1 and 106; between amino acid positions 1 and 111; between amino acid positions 1 and 116; between amino acid positions 1 and 121; or between amino acid positions 1 and 126 of SEQ ID NO: 32. In some cases, a truncated collagen as described herein may comprise a C-terminal truncation at any amino acid position between amino acid positions 276 and 1366; between amino acid positions 281 and 1366; between amino acid positions 286 and 1366; between amino acid positions 291 and 1366; between amino acid positions 296 and 1366; between amino acid positions 301 and 1366; or between amino acid positions 306 and 1366 of SEQ ID NO: 32. In some cases, a truncated collagen as described herein may comprise both an N-terminal truncation and a C-terminal truncation. For example, a truncated collagen as described herein may comprise an N-terminal truncation at any amino acid position between amino acid positions 1 and 101; between amino acid positions 1 and 106; between amino acid positions 1 and 111; between amino acid positions 1 and 116; between amino acid positions 1 and 121; or between amino acid positions 1 and 126 of SEQ ID NO: 32; and a C-terminal truncation at any amino acid position between amino acid positions 276 and 1366; between amino acid positions 281 and 1366; between amino acid positions 286 and 1366; between amino acid positions 291 and 1366; between amino acid positions 296 and 1366; between amino acid positions 301 and 1366; or between amino acid positions 306 and 1366 of SEQ ID NO: 32. In a specific embodiment, a truncated collagen as provided herein may comprise an N-terminal truncation at amino acid position 111 of SEQ ID NO: 32; and a C-terminal truncation at amino acid position 291 of SEQ ID NO: 32.

[0081] In some cases, a truncated collagen as described herein may comprise an internal truncation at any amino acid position between amino acid positions 16 and 240; between amino acid positions 16 and 245; between amino acid positions 16 and 250; between amino acid positions 16 and 255; between amino acid positions 16 and 260; between amino acid positions 16 and 265; between amino acid positions 6 and 255; between amino acid positions 11 and 255; between amino acid positions 21 and 255; between amino acid positions 26 and 255; between amino acid positions 31 and 255; between amino acid positions 21 and 250; between amino acid positions 21 and 245; between amino acid positions 26 and 250; between amino acid positions 26 and 245; between amino acid positions 31 and 250; or between amino acid positions 31 and

245 of SEQ ID NO: 33. In a specific embodiment, a truncated collagen as described herein may comprise an internal truncation at amino acid positions 16 and 255 of SEQ ID NO: 33.

[0082] In some cases, a truncated collagen may comprise any amino acid sequence provided in Table 2 below. In some cases, a truncated collagen may consist of any amino acid sequence provided in Table 2 below. In some cases, a truncated collagen may consist essentially of any amino acid sequence provided in Table 2 below. In specific embodiments, the non-naturally occurring collagen is or comprises an amino acid sequence of any one of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29. In some embodiments, the truncated collagen comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, or at least 98% sequence identity to any one of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29.

Table 2. Non-limiting examples of truncated collagens

SEQ ID NO:	Amino acid sequence
SEQ ID NO: 2	MKKIWLALAGLVLAFSASAAQYEDHHHHHHHHHSGSSSLVPRGSHM GPQGVVGADGKDGTPEGEKGEQGRGAAGKQGSPPGADGARGPLGSI GQQGARGEPGDPGSPGLRGDTGLAGVKGVAGPSGRPGQPGANGLP GVNGRGGGLERGLAGPPGPDGRRGETGSPGIAGALGKPGLEGPKGY PGLRGRDGTNGKRGEQGETGPDGVRGIPGNDGQSGKPGIDGIDGT NGQPGEAGYQGGRGTRGQLGETGDVGQNGDRGAPGPDGSKGSAGR PGLR
SEQ ID NO: 4	GPQGVVGADGKDGTPEGEKGEQGRGAAGKQGSPPGADGARGPLGSI GQQGARGEPGDPGSPGLRGDTGLAGVKGVAGPSGRPGQPGANGLP GVNGRGGGLERGLAGPPGPDGRRGETGSPGIAGALGKPGLEGPKGY PGLRGRDGTNGKRGEQGETGPDGVRGIPGNDGQSGKPGIDGIDGT NGQPGEAGYQGGRGTRGQLGETGDVGQNGDRGAPGPDGSKGSAGR PGLR
SEQ ID NO: 7	MKKIWLALAGLVLAFSASAAQYEDGPQGVVGADGKDGTGNAGQK GPSGEPGSPGKAGSAGEQGPPGKDGSNGEPGSPGKEGERGLAGPP GPDGRRGETGSPGIAGALGKPGLEGPKGYPLRGRDGTNGKRGEQ GETGPDGVRGIPGNDGQSGKPGIDGIDGTNGQPGEAGYQGGRGTR GQLGETGDVGQNGDRGAPGPDGSKGSAGRPLR
SEQ ID NO: 5	GPQGVVGADGKDGTGNAGQKGPSGEPGSPGKAGSAGEQGPPGKD GSNGEPGSPGKEGERGLAGPPGPDGRRGETGSPGIAGALGKPGLE GPKGYPLRGRDGTNGKRGEQGETGPDGVRGIPGNDGQSGKPGID GIDGTNGQPGEAGYQGGRGTRGQLGETGDVGQNGDRGAPGPDGSK GSAGRPLR
SEQ ID NO: 10	MKKIWLALAGLVLAFSASAAQYEDHHHHHHHHHSGSSSLVPRGSHM SGSSSKGEELFTGVVPIILVELDGDVNGHKFSVRGEGEGDATNGKL TLKFICTTGKLPVPWPTLVTTLTLYGVQCFSRYPDHMKQHDFFKSA

	MPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKGIDFKE DGNILGHKLEYNFNHNVYITADKQKNGIKANFKIRHNVEDGVSQ LADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMLLE FVTAAGITHGMDELYKSGAPGGPQGVVGADGKDGTGPNAGQKGPS GEPGSPGKAGSAGEQGPPGKDGSGNGEPGSPGKEGERLAGPPGPD GRRGETGSPGIAGALGKPGLEGPKGYPLRGRDGTNGKRGEQGET GPDGVRGIPGNDGQSGKPGIDGIDGTNGQPGEAGYQGGRTGRQL GETGDVGQNGDRGAPGPDGSKGSAGRPLRHPETLVKVKDAEDQL GARVGYIELDLNSGKILESFRPEERFPMMSSTFKVLLCGAVLSRID AGQEQLGRRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMS DNTAANLLLTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPN DERDRTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTG SQATMDERNRQIAEIGASLIKH
SEQ ID NO: 12	MKKIWLALAGLVLAFAASAAQYEDHHHHHHHHHSGSSSLVPRGSHM SGSSSKGEELFTGVVPII LVELDGDVNGHKFSVRGEGEGDATNGKL TLKFICTTGKLPVPWPTLVTTLTLYGVQCFSRYPDHMKQHDFKSA MPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKGIDFKE DGNILGHKLEYNFNHNVYITADKQKNGIKANFKIRHNVEDGVSQ LADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMLLE FVTAAGITHGMDELYKSGAPGGPQGVVGADGKDGTGPNAGQKGPS GEPGSPGKAGSAGEQGPPGKDGSGNGEPGSPGKEGERLAGPPGPD GRRGETGSPGIAGALGKPGLEGPKGYPLRGRDGTNGKRGEQGET GPDGVRGIPGNDGQSGKPGIDGIDGTNGQPGEAGYQGGRTGRQL GETGDVGQNGDRGAPGPDGSKGSAGRPLRHPETLVKVKDAEDQL GARVGYIELDLNSGKILESFRPEERFPMMSSTFKVLLCGAVLSRID AGQEQLGRRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMS DNTAANLLLTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPN DERDRTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTG SQATMDERNRQIAEIGASLIKH
SEQ ID NO: 14	MKKIWLALAGLVLAFAASAAQYEDAGFPGLPGPAGEPGRHGKDG MGSPGFKGEAGSPGAPGQDGTGRGEPGIPGFPGNRGLMGQKGEIGP PGQQGKKGAPGMPGLMGSNGSPGQPGTPGSKGSKGEPGIQGMPGA SGLKGEPEGATGSPGEPGYMGLPGIQGKKGDKGNQGEKGIQGGKGE NGRQGI PGQQGIQGHGAKGERGEKGE PGVR
SEQ ID NO: 16	AGFPGLPGPAGEPGRHGKDGMLMGSPGFKGEAGSPGAPGQDGTGR PGIPGFPGNRGLMGQKGEIGPPGQQGKKGAPGMPGLMGSNGSPGQ PGTPGSKGSKGEPGIQGMPGA SGLKGEPEGATGSPGEPGYMGLPGI QGKKGDKGNQGEKGIQGGKGENGRQGI PGQQGIQGHGAKGERGE KGE PGVR
SEQ ID NO: 18	MKKIWLALAGLVLAFAASAAQYEDMGPPGSRGASGPAGVRGPNGD AGRPGEPLMGPRGLPGSPGNIGPAGKEGPVGLPGIDGRPGPIGP AGARGE PGNIGFP GPKGPTGDPGKNGDKGHAGLAGARGAPGPDGN NGAQPPGPQGVQGGKGEQGPAGPPGFQGLPGPSGPAGEVGKPGE RGLHGEFGLPGPAGPRGERGPPGESGAAGPTG
SEQ ID NO: 20	MGPPGSRGASGPAGVRGPNGDAGRPGEPLMGPRGLPGSPGNIGP AGKEGPVGLPGIDGRPGPIGPAGARGE PGNIGFP GPKGPTGDPGK NGDKGHAGLAGARGAPGPDGNNGAQPPGPQGVQGGKGEQGPAGP PGFQGLPGPSGPAGEVGKPGERGLHGEFGLPGPAGPRGERGPPGE SGAAGPTG

SEQ ID NO: 22	MKKIWLALAGLVLAFSASAAQYEDGFQGPAGEPGEPPGQTGPAGAR GPAGPPGKAGEDGHPGKPRPGERGVVGPQGARGFPGTPLPGFK GIRGHNGLDGLKGQPGAPGVKGEPGAPGENGTPGQTGARGLPGER GRVGAPGPAGARGSDGSVGPVGPAGPIGSAGPPGFPAGPGPKGEI GAVGNAGPAGPAGPRGEVGLPGL
SEQ ID NO: 24	GFQGPAGEPGEPPGQTGPAGARGPAGPPGKAGEDGHPGKPRPGER GVVGPQGARGFPGTPLPGFKGIRGHNGLDGLKGQPGAPGVKGEP GAPGENGTPGQTGARGLPGERGRVGAPGPAGARGSDGSVGPVGP GPIGSAGPPGFPAGPGPKGEI GAVGNAGPAGPAGPRGEVGLPGL
SEQ ID NO: 25	MKKIWLALAGLVLAFSASAGDQGPVGRGTGEVAVGPPGFAGEKGP SGEAGTAGPPGTGPGQGLLGAPGILGLPGSRGERGLPGVAVAGE PGPLGIAGPPGARGPPGAVGSPGVNAPGEAGRDGNPNDGPPGR DQQPGHKGERGYPGNIGPVGAAAGAPGHPGVPAGKHGNGRGETGP SGPVGPAGAVGPRGPSGPQGIRGDKGEPGEKGPRLPGLGDYKDD DDK
SEQ ID NO: 27	MKKIWLALAGLVLAFSASAKGHNGLQGLPGIAGHHGDQAGPSVG PAGPRGPAGPSGPAGKDGRGTGHPGTVPAGIRGPQGHQGPAGPPG PPGPPGPPGVSGGGYDFGYDGFYRADQPRSAPSLRPKDYEVDAT LKSLNNQIETLLTPEGSRKNPARTCRDLRLSHPEWSSGYYWIDPN QGCTMDAIKVYCDFSTGETCIRAQPENIPAKNWYRSSKDGDKDD DDK
SEQ ID NO: 29	MKKIWLALAGLVLAFSASAYEVDATLKSLNNQIETLLTPEGSRK PARTCRDLRLSHPEWSSGYYWIDPNQGCTMDAIKVYCDFSTGETC IRAQPENIPAKNWYRSSKDKKHVWLGETINAGSQFEYNVEGVTSK EMATQLAFMRLLANYASQNIYHCKNSIAYMDEETGNLKKAVILQ GSNDVELVAEGNSRFTYTVLVDGCSKKTNEWGKTIIEYKTNKPSR LPFLDIAPLDIGGADQEFFVDIGPVCFKGDYKDDDDDK

[0083] In some cases, a truncated collagen may be between 100 and 300 amino acids, between 150 and 250 amino acids, between 160 and 250 amino acids, between 160 and 220 amino acids, between 170 and 200 amino acids, between 180 and 190 amino acids, or between 185 and 190 amino acids in length.

[0084] The non-naturally occurring collagen may, in some embodiments, further comprise amino acid sequences including a secretion tag. The secretion tag may direct the collagen to the periplasmic space of the host cell. In particular embodiments, the signal peptide is derived from DsbA, PelB, OmpA, TolB, MalE, lpp, TorA, HylA, DegP, or a hybrid secretion tag that comprises a portion of one secretion tag fused to a portion of a second secretion tag. In one aspect the secretion tag may be attached to the non-naturally occurring collagen. In another aspect the secretion tag may be cleaved from the non-naturally occurring collagen.

[0085] In some embodiments, the non-naturally occurring collagen comprises a histidine (or polyhistidine) tag. In specific embodiments, the histidine tag or polyhistidine tag is or comprises a sequence of 2 to 20 histidine residues that are attached to the collagen. In various embodiments, the histidine tag comprises 2 to 20 histidine residues, 5 to 15 histidine residues, 5

to 18 histidine residues, 5 to 16 histidine residues, 5 to 15 histidine residues, 5 to 14 histidine residues, 5 to 13 histidine residues, 5 to 12 histidine residues, 5 to 11 histidine residues, 5 to 10 histidine residues, 6 to 12 histidine residues, 6 to 11 histidine residues, or 7 to 10 histidine residues. The histidine tags may be useful in purification of proteins by chromatographic methods utilizing nickel based chromatographic media. Exemplary fluorescent proteins include green fluorescent protein (GFP) or red fluorescent protein (RFP). Fluorescent proteins are well known in the art. In one embodiment, a non-naturally occurring collagen comprises a GFP and/or RFP. In one embodiment, a superfolder GFP is fused to a non-naturally occurring collagen. The superfolder GFP may be a GFP that folds properly even when fused to a poorly folded polypeptide. In one aspect, a histidine tag may be attached to the non-naturally occurring collagen. In another aspect, a histidine tag may be cleaved from the non-naturally occurring collagen.

[0086] In some embodiments, the non-naturally occurring collagen further comprises a protease cleavage site. The protease cleavage site may be useful to cleave the recombinantly produced collagen to remove one or more portions of the polypeptide. The portions of the polypeptide that may be removed include the secretion tag, the histidine tag, the fluorescent protein tag, and/or the Beta-lactamase. The proteases may comprise endoproteases, exoproteases, serine proteases, cysteine proteases, threonine proteases, aspartic proteases, glutamic proteases, and metalloproteases. Exemplary protease cleavage sites include amino acids that are cleaved by Thrombin, TEV protease, Factor Xa, Enteropeptidase, and Rhinovirus 3C Protease. In one aspect, the cleavage tag is attached to the non-naturally occurring collagen. In another aspect, the cleavage tag is removed by an appropriate protease from the non-naturally occurring collagen.

[0087] In some embodiments, the non-naturally occurring collagen further comprises an enzyme that is a Beta-lactamase. The beta-lactamase may be useful as a selection marker. In one aspect, the beta-lactamase is attached to the non-naturally occurring collagen. In another aspect, the beta-lactamase is cleaved from the non-naturally occurring collagen.

[0088] Provided in certain embodiments herein are (e.g., topical) compositions or formulations comprising one or more polypeptide provided herein. In some embodiments, the composition provides any suitable amount of polypeptide provided herein, such as in any suitable amount (e.g., an amount suitable to provide a benefit when given or administered to an individual or cell). In some specific embodiments, the composition comprises an amount suitable to provide a beneficial effect to the skin of an individual when (e.g., topically) administered to the skin of the individual. In specific embodiments, the composition comprises between 0.001% and 30% w/w

of a polypeptide (or non-naturally occurring collagen) such as provided herein. In more specific embodiments, the composition comprises between 0.001% and 20% w/w of a polypeptide (or non-naturally occurring collagen) such as provided herein, between 0.001% and 10% w/w of a polypeptide (or non-naturally occurring collagen) such as provided herein, between 0.001% and 5% w/w of a polypeptide (or non-naturally occurring collagen) such as provided herein, between 0.001% and 2% w/w of a polypeptide (or non-naturally occurring collagen) such as provided herein, between 0.001% and 1% w/w of a polypeptide (or non-naturally occurring collagen) such as provided herein, between 0.001% and 0.5% w/w of a polypeptide (or non-naturally occurring collagen) such as provided herein, and between 0.001% and 0.2% w/w of a polypeptide (or non-naturally occurring collagen) such as provided herein.

[0089] In one aspect, the compositions that comprise non-naturally occurring collagen may be personal care products (e.g., a cosmetic). In some embodiments, the compositions are formulated for topical administration. The compositions can contain other cosmetic ingredients suitable for human use. The personal care products may be useful for preventing or treating ultraviolet radiation damage to human skin or hair. The personal care products may be useful for increasing the firmness, elasticity, brightness, hydration, tactile texture or visual texture of skin and/or stimulate collagen production. The personal care products may be useful for reducing redness of the skin. The personal care products may be applied to skin or hair. The compositions include, for example, masks, skin cleaners such as soap, cleansing creams, cleansing lotions, facial cleansers, cleansing milks, cleansing pads, facial washes, facial and body creams and moisturizers, facial serums, facial and body masks, facial toners and mists, eye creams and eye treatments, exfoliator formulas, lip balms and lipsticks, hair shampoo, hair conditioner and body shampoos, hair and scalp serums, hair mists and sprays, eye shadow, concealer, mascara and other color cosmetics.

[0090] The compositions that comprise the non-naturally occurring collagen can further comprise at least one additional ingredient comprising a topical carrier or a preservative. The topical carrier may comprise a topical carrier selected from the group consisting of liposome, biodegradable microcapsule, lotion, spray, aerosol, dusting powder, biodegradable polymer, mineral oil, triglyceride oil, silicone oil, glycerin, glycerin monostearate, alcohols, emulsifying agents, liquid petroleum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene, wax, sorbitan monostearate, polysorbate, cetyl ester wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol, cyclomethicone, cyclopentasiloxane and water. The preservative may comprise a preservative selected from the group consisting of tocopherol, diiodomethyl-p-tolylsulfone, 2-Bromo-2-nitropropane-1,3-diol, cis isomer 1-(3-chloroallyl)-

3,5,7-triaza-1-azoniaadamantane chloride, glutaraldehyde, 4,4-dimethyl oxazolidine, 7-Ethylbicyclooxazolidine, phenoxyethanol, butylene glycol, 1,2 Hexanediol, methyl paraben, sorbic acid, Germaben II, rosemary extract, and EDTA

[0091] Also provided in certain embodiments herein, are methods of decreasing skin damage, promoting the repair of damaged skin, protecting skin against UV damage, and/or protecting skin cells against the effects of exposure to urban dust. In another embodiment, methods of increasing the firmness, elasticity, brightness, hydration, tactile texture, or visual texture of skin and/or stimulating collagen production are provided. The methods may comprise a step of applying a composition comprising a non-naturally occurring collagen to the skin of a subject. Without being bound to a particular theory or mechanism, the collagen in the composition may decrease skin damage by protecting against UV damage. In some cases, the collagen in the composition may promote the repair of damaged skin by increasing the viability of cells. In some cases, the collagen in the composition may decrease skin damage and/or promote repair of cells by increasing procollagen synthesis when applied to skin, and/or promoting the viability of skin cells. In some cases, the collagens decrease the formation of thymine-thymine (TT) dimer formation.

[0092] The methods provided herein encompass the use of a composition for treatment indicated in the method, such as by the steps provided herein. In embodiments, the disclosure provides the use of a composition provided herein (e.g., a truncated collagen or a formulation comprising a truncated collagen) in a method for decreasing skin damage, promoting the repair of damaged skin, protecting skin against UV damage, and/or protecting skin cells against the effects of exposure to urban dust (e.g., such as by administering to the skin of a subject a composition provided herein). In embodiments, the disclosure provides the use of a composition provided herein (e.g., a truncated collagen or a formulation comprising a truncated collagen) in a method for increasing the firmness, elasticity, brightness, hydration, tactile texture, or visual texture of skin and/or stimulating collagen production.

[0093] In some embodiments, a truncated collagen as provided herein may stimulate fibroblast and/or keratinocyte production of collagen type I (see, e.g., Example 4 and Example 6). In some cases, the levels of pro-collagen type I C-peptide (a read-out for collagen production) may be measured. In some cases, an *in vitro* MatTek full thickness human skin tissue model may be used (see, e.g., Example 6) to assess pro-collagen type I C-peptide levels. In some cases, collagen type I levels may be measured or determined by an enzyme-linked immunosorbent assay (ELISA). In some cases, a truncated collagen as provided herein may stimulate production

of collagen type I at a higher level than untreated cells, cells treated with retinol, and/or cells treated with Vitamin B3.

[0094] In some embodiments, a truncated collagen as provided herein may stimulate fibroblast overexpression of extracellular matrix genes (see, e.g., Example 4). In some cases, the levels of extracellular matrix genes may be measured by RNA sequencing. In some cases, a truncated collagen as provided herein may stimulate fibroblast overexpression of one or more of the collagen type I gene (COL1A), the elastin gene (ELN), and the fibronectin gene (FN1). In some cases, the levels of extracellular matrix genes produced by fibroblasts treated with a truncated collagen provided herein may be higher than untreated fibroblasts, or fibroblasts treated with retinol. In some cases, the levels of extracellular matrix genes produced by fibroblasts treated with a truncated collagen provided herein may be similar to, or higher than, fibroblasts treated with Vitamin C.

[0095] In some embodiments, a truncated collagen as provided herein may reduce inflammation of keratinocytes irradiated with UVB light (see, e.g., Example 4 and Example 6). In some cases, keratinocytes may be irradiated with UVB light, and then treated with a truncated collagen as provided herein. In some cases, inflammation may be measured by measuring the levels of IL-1 α produced by UVB-irradiated keratinocytes (e.g., by ELISA). In some cases, UVB-irradiated keratinocytes may produce lower levels of IL-1 α when treated with a truncated collagen provided herein than untreated keratinocytes.

[0096] In some embodiments, a truncated collagen as provided herein may increase viability of keratinocytes irradiated with UVB light (see, e.g., Example 4). In some cases, keratinocytes may be pre-treated (prior to UVB irradiation) and post-treated (after UVB irradiation) with a truncated collagen provided herein. In some cases, cell viability may be measured using an MTT metabolic colorimetric assay. In some cases, keratinocytes treated with a truncated collagen provided herein may exhibit greater cell viability after UVB irradiation than untreated keratinocytes.

[0097] In some embodiments, a truncated collagen as provided herein may reduce DNA damage in keratinocytes after exposure to UVB light (see, e.g., Example 6). In some cases, DNA damage may be assessed by measuring the levels of thymine dimers (TT-dimers). In a non-limiting example, the OxiSelect UV-induced DNA damage ELISA kit may be used to measure TT-dimer levels. In some cases, UVB-irradiated keratinocytes treated with a truncated collagen provided herein may show lower levels of TT-dimers than untreated keratinocytes.

[0098] In some embodiments, a truncated collagen as provided herein may have anti-oxidative capacity (see, e.g., Example 4 and Example 6). In some cases, an oxygen radical absorbance

capacity (ORAC) assay may be used to measure oxidative capacity of the truncated collagen. In a non-limiting example, a truncated collagen in the form of a 0.1% solution may have anti-oxidative properties of at least 10 μ M Trolox (Vitamin E) equivalents (TEs), at least 50 μ M TEs, at least 100 μ M TEs, at least 150 μ M TEs, at least 160 μ M TEs, at least 170 μ M TEs, at least 180 μ M TEs, at least 190 μ M TEs, or at least 200 μ M TEs.

[0099] In some embodiments, a truncated collagen as provided herein may increase cell viability of keratinocytes exposed to urban dust pollution as compared to untreated cells (see, e.g., Example 6). In some cases, cell viability may be measured by an MTT metabolic colorimetric assay.

[0100] In some embodiments, topical administration of a truncated collagen provided herein to the face of a subject may result in increased facial skin elasticity, as compared to baseline, at 1 week, 2 weeks, 4 weeks, 8 weeks, or longer, post-treatment (see, e.g., Example 5 and Example 7). In some cases, facial skin elasticity may be measured by a cutometer.

[0101] In some embodiments, topical administration of a truncated collagen provided herein to the face of a subject may result in an increase in facial skin collagen content, as compared to baseline, at 1 week, at 2 weeks, at 4 weeks, at 8 weeks, or longer, post-treatment (see, e.g., Example 5). In some cases, facial skin collagen content may be measured by a SIAscope.

[0102] In some embodiments, topical administration of a truncated collagen provided herein may result in a reduction in facial skin redness (erythema), as compared to baseline, at 1 week, at 2 weeks, at 4 weeks, at 8 weeks, or longer, post-treatment (see, e.g., Example 5). In some cases, facial skin redness (erythema) may be scored by a blinded clinical grader (e.g., using a 5-point ordinal scale as provided in Table 4).

[0103] In some embodiments, topical administration of a truncated collagen provided herein may result in a reduction in facial wrinkles, as compared to baseline, at 1 week, at 2 weeks, at 4 weeks, at 8 weeks, or longer, post-treatment (see, e.g., Example 5). In some cases, facial wrinkles may be scored by a blinded clinical grader.

[0104] In some embodiments, topical administration of a truncated collagen provided herein may result in increased facial skin moisture, as compared to baseline, at 1 week, at 2 weeks, at 4 weeks, at 8 weeks, or longer, post-treatment (see, e.g., Example 7). In some cases, topical administration of a truncated collagen provided herein may result in increased facial skin moisture as compared to topical administration of a marine collagen. In some cases, skin hydration may be measured by a corneometer.

[0105] One aspect of this disclosure provides polynucleotides that encode a non-naturally occurring collagen. The polynucleotides may encode collagen from jellyfish or human. The

polynucleotides may encode for a collagen that is full length or truncated. In various embodiments, the polynucleotide may comprise a polynucleotide according to any one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 26, SEQ ID NO: 28, or SEQ ID NO: 30, or a homolog thereof (e.g., having at least 85%, at least 90%, at least 95%, or at least 98% sequence identity thereto). In some cases, the polynucleotide may be codon optimized (e.g., for expression in a host cell).

[0106] In another aspect the present disclosure provides polynucleotides that encode collagen fusion proteins. The collagen fusion proteins may comprise a secretion tag, a histidine tag, a fluorescent protein tag, a protease cleavage site, a Beta-lactamase along and/or GEK amino acid trimer repeats and/or GDK amino acid trimer repeats together with collagen.

[0107] In an aspect, vectors comprising the collagen encoding polynucleotides may be used to transform host cells and express the polynucleotides. The polynucleotides may further comprise nucleic acids that encode enzymes that permit the host organism to grow in the presence of a selection agent. The selection agents may include certain sugars including galactose containing sugars or antibiotics including ampicillin, hygromycin, G418, and others. Enzymes that can be used to confer resistance to the selection agent include β -galactosidase or a β -lactamase.

[0108] In one aspect, host cells that express the polynucleotides of the invention are provided. Host cells can be any host cell including gram negative bacterial cells, gram positive bacterial cells, yeast cells, insect cells, mammalian cells, plant cells, or any other cells used to express exogenous polynucleotides. An exemplary gram-negative host cell is *E. coli*.

[0109] Any desirable or necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. In certain embodiments, the medium further comprises one or more ingredients selected from: ammonium chloride, ammonium sulfate, calcium chloride, casamino acids, iron(II) sulfate, magnesium sulfate, peptone, potassium phosphate, sodium chloride, sodium phosphate, and yeast extract.

[0110] Beta-lactamases are enzymes that confer resistance to lactam antibiotics in prokaryotic cells. Typically when Beta-lactamases are expressed in bacterial host cells, the expressed Beta-lactamase proteins also include targeting sequences (secretion tag) that direct the Beta-lactamase proteins to the periplasmic space. Beta-lactamases are not functional unless they are transported to the periplasmic space. Beta-lactamases targeted to the periplasmic space without the use of an independent secretion tag that targets the enzyme to the periplasmic space are provided. By creating a fusion protein in which a periplasmic secretion tag is added to the N-terminus of a

protein such as GFP, collagen, or GFP/collagen chimeras, the functionality of the Beta-lactamase lacking a native secretion tag can be used to select for full translation and secretion of the N-terminal fusion proteins. Using this approach, a DsbA-GFP-Collagen-Beta-lactamase fusion may be used to select for truncation products in the target collagens that favor translation and secretion.

[0111] Another embodiment provides methods of producing a polypeptide (or non-naturally occurring collagen), such as provided herein. In some embodiments, the method comprises the steps of inoculating a culture medium with a recombinant host cell comprising polynucleotides that encode the polypeptide or “collagen,” cultivating the host cell, and isolating the polypeptide (or non-naturally occurring collagen) from the host cell.

[0112] A process for fermentative preparation of a polypeptide (or protein) is provided. The process comprises the steps of:

(a) culturing a recombinant Gram-negative bacterial cell in a medium comprising a magnesium salt, wherein the concentration of magnesium ions in the medium is at least about 6 mM, and wherein the bacterial cell comprises an exogenous gene encoding the protein; and

(b) harvesting the protein from the medium.

[0113] The bacteria may be cultured in any suitable manner, such as continuously—as described, for example, in WO 05/021772—or discontinuously in a batch process (batch cultivation) or in a fed-batch or repeated fed-batch process for the purpose of producing the target protein. In some embodiments, protein production is conducted on a large-scale. Various large-scale fermentation procedures are available for production of recombinant proteins. Large-scale fermentations have at least 1,000 liters of capacity, preferably about 1,000 to 100,000 liters of capacity. In some instances, fermenters use agitator impellers to distribute oxygen and nutrients, especially glucose (the preferred carbon/energy source). Small-scale fermentation refers generally to fermentation in a fermenter that is no more than approximately 20 liters in volumetric capacity.

[0114] For accumulation of the target protein, the host cell may be cultured under conditions sufficient for accumulation of the target protein. Such conditions include, e.g., temperature, nutrient, and cell-density conditions that permit protein expression and accumulation by the cell. Moreover, such conditions may be those under which the cell can perform basic cellular functions of transcription, translation, and passage of proteins from one cellular compartment to another for the secreted proteins, as are known to those skilled in the art.

[0115] Any suitable bacterial cell is optionally utilized in a method provided herein. The bacterial cells may be cultured at any suitable temperature. In specific embodiments, the bacterial cells are *E. coli* cells. For *E. coli* growth, for example, the typical temperature ranges from about 20° C to about 39° C. In one embodiment, the temperature is from about 20° C to about 37° C. In another embodiment, the temperature is at about 30° C. In one embodiment, the host cells, in the non-switched state or switched state may be cultivated at one temperature and switched to a different temperature to induce protein production. The host cells may be cultivated first at one temperature to propagate the cells, then to induce protein production the cells may be cultivated at a lower temperature. The first temperature may be about 23°, 24°, 25°, 26°, 27°, 28°, 29°, 30°, 31°, 32°, 33°, 34°, 35°, 36°, or 37° C. The second temperature may be about 20°, 21°, 22°, 23°, 24°, 25°, 26°, 27°, 28°, 29°, 30°, 31°, 32°, 33°, 34°, 35° or 36° C. The cultivation at the second temperature may be conducted between 1 hour and 100 hours, between 5 hours and 90 hours, between 5 hours and 80 hours, between 5 hours and 80 hours, between 5 hours and 70 hours, between 10 hours and 70 hours, between 15 hours and 70 hours, between 15 hours and 65 hours, between 15 hours and 60 hours, between 20 hours and 60 hours, between 20 hours and 55 hours, between 20 hours and 50 hours, between 24 hours and 50 hours, between 24 hours and 48 hours, between 30 hours and 50 hours, between 30 hours and 45 hours, or between 30 hours and 40 hours.

[0116] The pH of the culture medium may be any pH from about 5-9, depending mainly on the host organism. For *E. coli*, the pH may be from about 6.0 to about 7.4, about 6.2 to about 7.2, about 6.2 to about 7.0, about 6.2 to about 6.8, about 6.2 to about 6.6, about 6.4 or about 6.5.

[0117] For induction of gene expression, typically the cells may be cultured until a certain optical density is achieved, e.g., an OD₆₀₀ of about 1.1, at which point induction is initiated (e.g., by addition of an inducer, by depletion of a repressor, suppressor, or medium component, etc.) to induce expression of the exogenous gene encoding the target protein. In some embodiments, expression of the exogenous gene may be inducible by an inducer selected from, e.g., isopropyl-β-d-1-thiogalactopyranoside, lactose, arabinose, maltose, tetracycline, anhydrotetracycline, vavlycin, xylose, copper, zinc, and the like. The induction of gene expression can also be accomplished by decreasing the dissolved oxygen levels during fermentation. The dissolved oxygen levels of the fermentation during cell propagation may be between 10% and 30%. To induce gene expression the dissolved oxygen level may be reduced to below 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0%. In host cells, in either the physiological state or the switched state, protein production can be induced by lowering the temperature of the fermentation as disclosed herein.

EXAMPLES

Example 1. Production of Truncated Collagen

[0118] A codon optimized DNA sequence, optimized for expression in *E. coli*, encoding a jellyfish collagen with a truncation of 240 internal amino acids (relative to full-length jellyfish collagen (SEQ ID NO: 33)) was synthesized and expressed. The DNA sequence is shown below in SEQ ID NO: 1. In SEQ ID NO: 1, the DsbA secretion tag is encoded by nucleotides 1-72 and encodes amino acids 1-24 of SEQ ID NO: 2. The histidine tag comprising 9 histidine residues is encoded by nucleotides 73-99 of SEQ ID NO: 1 and encodes amino acids 25-33 of SEQ ID NO: 2. The linker is encoded by nucleotides 100-111 of SEQ ID NO: 1 and encodes amino acids 34-37 of SEQ ID NO: 2. The thrombin cleavage site is encoded by nucleotides 112-135 of SEQ ID NO: 1 and encodes amino acids 38-45 of SEQ ID NO: 2. The truncated collagen is encoded by nucleotides 136-822 of SEQ ID NO: 1 and encodes amino acids 46-274 of SEQ ID NO: 2.

[0119] ATGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATC
GGCGGCGCAGTATGAAGATCACCATCACCACCACCATCACCCTCTGGCTCGA
GCCTGGTGCCGCGCGGCAGCCATATGGGTCCGCAGGGTGTTGTTGGTGCAGATGGT
AAAGACGGTACCCCGGGTGAAAAAGGAGAACAGGGACGTACAGGTGCAGCAGGTA
AACAGGGCAGCCCGGGTGCCGATGGTGCCCGTGGCCCGCTGGGTAGCATTGGTCAG
CAGGGTGCAAGAGGCGAACCGGGCGATCCGGGTAGTCCGGGCCTGCGTGGTGATAC
GGGTCTGGCCGGTGTTAAAGGCGTTGCAGGTCCTTCAGGTCGTCCAGGTCAACCGGG
TGCAAATGGTCTGCCGGGTGTTAATGGTCGTGGCGGTCTGGAACGTGGTCTGGCAGG
ACCGCCGGGTCCTGATGGTCGCCGCGGTGAAACGGGTTCACCGGGTATTGCCGGTG
CCCTGGGTAAACCAGGTCTGGAAGGTCCGAAAGGTTATCCTGGTCTGCGCGGTCTGTC
ATGGTACCAATGGCAAACGTGGCGAACAGGGCGAAACCGGTCCAGATGGTGTTCGT
GGTATTCGGGTAAACGATGGTCAGAGCGGTAAACCGGGCATTGATGGTATTGATGG
CACCAATGGTCAGCCTGGCGAAGCAGGTTATCAGGGTGGTCGCGGTACCCGTGGTC
AGCTGGGTGAAACAGGTGATGTTGGTCAGAATGGTGATCGCGGCGCACCGGGTCCG
GATGGTAGCAAAGGTAGCGCCGGTCGTCCGGGTTTACGTAA (SEQ ID NO: 1)

[0120] The truncated collagen is approximately 54% of the full length jellyfish collagen (SEQ ID NO: 33) and is disclosed below in SEQ ID NO: 2.

[0121] MKKIWLALAGLVLAFAASAAQYEDHHHHHHHHHSGSSLVPRGSHMGPQGVVG
ADGKDGPGEKGEGRTGAAGKQGSPPADGARGPLGSIGQQGARGEPPGDPGSPGLRG
DTGLAGVKGVAGPSRPGQPGANGLPGVNGRGGLERGLAGPPGPDGRRGETGSPGIAG
ALGKPGLEGPKGYPLRGRDGTNGKRGEQGETGPDGVRGIPGNDGQSGKPGIDGIDGT

NGQPGEAGYQGGRGTRGQLGETGDVGQNGDRGAPGPDGSKGSAGRPGLR (SEQ ID NO: 2)

[0122] The polynucleotide encoding the truncated jellyfish collagen without the DsbA secretion tag, the histidine tag, linker and thrombin cleavage site is disclosed in SEQ ID NO: 3.

[0123] GTCCGCAGGGTGTGTTGGTGCAGATGGTAAAGACGGTACCCCGGGTGAAAA
AGGAGAACAGGGACGTACAGGTGCAGCAGGTAAACAGGGCAGCCCGGGTGCCGAT
GGTGCCCGTGGCCCGCTGGGTAGCATTGGTCAGCAGGGTGCAAGAGGCGAACCGGG
CGATCCGGGTAGTCCGGGCCTGCGTGGTGATACGGGTCTGGCCGGTGTAAAGGCG
TTGCAGGTCTTCAGGTCTGCCAGGTCAACCGGGTGCAAATGGTCTGCCGGGTGTAA
ATGGTCTGTGGCGGTCTGGAACGTGGTCTGGCAGGACCGCCGGGTCTGATGGTCTGC
CGCGGTGAAACGGGTTCACCGGGTATTGCCGGTGCCCTGGGTAAACCAGGTCTGGA
AGGTCCGAAAGGTTATCTGGTCTGCGCGGTCTGTGATGGTACCAATGGCAAACGTG
GCGAACAGGGCGAAACCGGTCCAGATGGTGTTCGTGGTATTCCGGGTAAACGATGGT
CAGAGCGGTAAACCGGGCATTGATGGTATTGATGGCACCAATGGTCAGCCTGGCGA
AGCAGGTTATCAGGGTGGTCGCGGTACCCGTGGTCAGCTGGGTGAAACAGGTGATG
TTGGTCAGAAATGGTGATCGCGGCGCACCGGGTCCGGATGGTAGCAAAGGTAGCGCC
GGTCGTCCGGGTTTACGTAA (SEQ ID NO: 3)

[0124] The truncated jellyfish collagen amino acid sequence without the DsbA secretion tag, the histidine tag, linker and thrombin cleavage site is disclosed in SEQ ID NO: 4.

[0125] GPQGVVGADGKDGTGPEKGEQRTGAAGKQGSPGADGARGPLGSIGQQGARG
EPGDPGSPGLRGDTGLAGVKGVAGPSGRPGQPGANGLPGVNGRGGGLERGLAGPPGPDG
RRGETGSPGIAGALGKPGLEGPKGYPLGRGRDGTNGKRGEQGETGPDGVRGIPGNDGQ
SGKPGIDGIDGTNGQPGEAGYQGGRGTRGQLGETGDVGQNGDRGAPGPDGSKGSAGRP
GLR (SEQ ID NO: 4)

[0126] The polynucleotides of SEQ ID NO: 1 were codon optimized and synthesized by Gen9 DNA (now Ginkgo Bioworks) internal synthesis. Overlaps between the pET28 vector and SEQ ID NO: 1 were designed to be between 30 and 40 bp long and were added using PCR with the enzyme PrimeSTAR® GXL polymerase (www.clontech.com/US/Products/PCR/GC_Rich/PrimeSTAR_GXL_DNA_Polymerase?sitex=10020:22372:US). The opened pET28a vector and insert DNA (SEQ ID NO: 1) was then assembled together into the final plasmid using SGI Gibson Assembly® (us.vwr.com/store/product/17613857/gibson-assembly-hifi-1-step-kit-synthetic-genomics-inc). The plasmid sequence was then verified through Sanger sequencing through Eurofins Genomics (www.eurofinsgenomics.com).

[0127] The transformed cells were cultivated in minimal media and frozen in 1.5 ml aliquots with glycerol at a ratio of 50:50 of cells to glycerol. One vial of this frozen culture was revived in 50 ml of minimal media overnight at 37° C, 200 rpm. Cells were transferred into 300 ml of minimal media and grown for 6-9 hours to reach an OD600 of 5-10.

[0128] A bioreactor was prepared with 2.7 L of minimal media + glucose and 300 ml of OD600 of 5-10 culture was added to bring the starting volume to 3 L. Cells were grown at 28 °C, pH 7 with Dissolved Oxygen maintained at 20% saturation using a cascade containing agitation, air, and oxygen. pH was controlled using 28% w/w ammonium hydroxide solution. Fermentation was run in a fed-batch mode using a DO-stat based feeding algorithm once the initial bolus of 40 g/L was depleted around 13 hours. After 24-26 hours of initial growth, the OD600 reached above 100. At this point, 300 mL of 500 g/L sucrose was added and temperature was reduced to 25 °C. High density culture was induced for protein production using 1 mM IPTG. Fermentation was continued for another 20-24 hours and cells were harvested using a bench top centrifuge at 9000 rcf, 15° C for 60 minutes. The cell pellet recovered from centrifugation was resuspended in a buffer containing 0.5 M NaCl and 0.1 M KH₂PO₄ at pH 8 in a weight by weight ratio of 2x buffer to 1x cells.

[0129] The harvested cells were disrupted in a homogenizer at 14,000 psi pressure in 2 passes. The resulting slurry contained the collagen protein along with other proteins.

[0130] The fermentations were performed at various temperature ranging from 25° to 28° C. For some fermentations, the temperature of the fermentation was maintained at a constant temperature and immediately upon completion of fermentation (OD600 of 5-10) the collagen was purified. For other fermentations, the temperature of the fermentations was maintained for a desired period of time and when cell densities of OD600 of 5-10 were reached, the temperature was reduced to induce protein production. Typically, the temperature was reduced from 28° C to 25° C. After the fermentation at 25 °C was continued for 40-60 hours, the collagen was isolated.

[0131] The collagen was purified by acid treatment of homogenized cell broth. Additionally, acid treatment was also performed on non-homogenized whole cells recovered from the bioreactor after centrifugation and resuspension in the buffer described above. The pH of either the homogenized slurry or the resuspended whole cells was decreased to pH 3 using 6 M hydrochloric acid. Acidified cell slurry was incubated overnight at 4° C with mixing, followed by centrifugation. Supernatant of the acidified slurry was tested on a polyacrylamide gel and found to contain collagen in relatively high abundance compared to starting pellet. The collagen slurry thus obtained was high in salts. To obtain volume and salt reduction, concentration and diafiltration steps were performed using an EMD Millipore Tangential Flow Filtration system

with ultrafiltration cassettes of 0.1 m² each. Total area of filtration was 0.2 m² using 2 cassettes in parallel. A volume reduction of 5x and a salt reduction of 19x was achieved in the TFF stage. Final collagen slurry was run on an SDS-PAGE gel to confirm presence of the collagen. This slurry was dried using a multi-tray lyophilizer over 3 days to obtain a white, fluffy collagen powder.

[0132] The purified truncated collagen obtained from homogenized cell broth or non-homogenized cells were analyzed on an SDS-PAGE gel and a thick and clear band was observed at the expected size of 27 kilodaltons. The purified collagen was also analyzed by mass spectrometry and it was confirmed that the 27 kilodalton protein was jellyfish collagen.

[0133] An alternative purification method of the full length and truncated collagens is provided below.

[0134] The fermentation broth was mixed with 0.3-0.5% w/v of Poly Ethyl Imine (PEI). After 15 minutes of incubation with PEI, the fermentation broth was centrifuged at 9000 rcf for 15 minutes to recover the supernatant, which contained the collagen protein. The pellet containing the cells was discarded and the PEI-treated collagen containing supernatant was mixed with Sodium Bentonite (0.2% final w/v) (Wyopure®, Wyoming Bentonite) and centrifuged. The bentonite containing pellet was discarded and the supernatant was recovered.

[0135] The Bentonite treated supernatant was concentrated between 3-6 fold on a tangential flow filtration system (TFF) (EMD Millipore) using a 5 kDa cassette. The collagen was retained with almost no losses in the permeate stream. To remove salts, the retentate from the concentration step was diafiltered using the same TFF set-up. Final conductivity of the protein solution was < 10 milliSiemens. The typical conductivity was between 400 microsiemens and 1.5 millisiemens. Highly concentrated collagen solutions had higher conductivities approaching 4 milliSiemens. A skilled artisan will understand that conductivities higher than 10 milliSiemens may be observed depending on the concentration of the collagen. Next, the desalted and concentrated protein was subjected to treatment with activated carbon using the W-L 9000 10 x 40 granulated resin (Carbon Activated Corporation). 5% w/v of the carbon resin was mixed with collagen containing protein feed and mixed at 45-50° C with mild agitation. The carbon-treated slurry was filtered using a Buchner funnel lined with an Ertel Filter Press Pad M-953 (Ertel Alsop) in presence or absence of a filtration aid such as Diatomaceous Earth (Sigma Aldrich). Post-filtration, the collagen solution was filtered through a 0.2 micron filter followed by one to several hours of treatment with Sodium Bentonite (0.2% w/v final) (Wyopure®, Wyoming Bentonite) and centrifuged at 9000 rcf, 15-30 minutes to obtain a highly pure, clear and particulate free collagen solution. When removal of endotoxin proteins was desired, the protein was passed through a

chromatographic filter like Sartobind-Q (Sartorius-Stedim) to specifically remove endotoxin proteins.

[0136] The purified collagen was analyzed on an SDS-PAGE gel and a thick and clear band was observed at 30 kilodaltons. The upshift in size is due to the structure of the collagen molecule and the high glycine/proline amino acid content. The purified collagen was also analyzed by mass spectrometry and it was confirmed that the 30 kilodalton protein was the truncated collagen.

[0137] The truncated collagens were further analyzed by HPLC using an Agilent 1100 series HPLC. The column was the 50 mm Agilent PLRP-S reverse phase column with an inner diameter of 4.6 mm, μ M particle size and 1000 Angstrom pore size.

[0138] The sample was prepared by diluting 1:1 in a 0.04% sodium azide solution in HPLC-grade water. After dilution, the resulting mixture was filtered through a 0.45 μ m filter to remove any large particles that can clog the HPLC column. For analysis, the samples are diluted appropriately with a 20 mM ammonium acetate buffer in HPLC-grade water at a pH of about 4.5. After mixing the sample, it was transferred to a 300 μ L microvial that was then placed in the autosampler. Using ChemStation, the software that operates the HPLC, the analysis parameters such as sample flowrate, column temperature, mobile phase flowrate, mobile phase composition, etc. can be altered. In one exemplary, but non-limiting analysis the parameters were: sample flow rate of 1 mL/min, column temperature of 80° C, column pressure of 60-70 bar, mobile phase composition of 97.9% water/1.9% acetonitrile with 0.2% trifluoroacetic acid; UV wavelength for analysis of 214.4 nm, injection volume of 10 μ L, and sample run time of 10 minutes.

[0139] Under these conditions, the truncated jellyfish collagen of SEQ ID NO: 5 has an elution time of about 5.4 minutes. ChemStation quantifies the peak area of the elution peak and calculates the protein concentration using a calibration curve that directly relates peak area to protein concentration. The calibration curve is generated using a known collagen solution that is serially diluted to contain collagen concentration ranges of 0.06 mg/mL to 1.00 mg/mL.

Truncated Collagen Without His Tag-Linker-Thrombin Cleavage Site

[0140] A truncated jellyfish collagen without a His tag, linker, and thrombin cleavage site is disclosed below. The codon-optimized nucleotide sequence encoding this collagen is provided in SEQ ID NO: 6. The amino acid sequence is disclosed in SEQ ID NO: 7. The DsbA secretion tag is encoded by nucleotides 1-72 of SEQ ID NO: 6 and encodes amino acids 1-24 of SEQ ID NO: 7. The truncated collagen sequence is encoded by nucleotides 73-639 of SEQ ID NO: 6 and encodes amino acids 25-213 of SEQ ID NO: 7.

[0141] ATGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATC
GGCGGCGCAGTATGAAGATGGTCCGCAGGGTGTGTTGGTGCAGATGGTAAAGACG
GTACCCCGGGTAATGCAGGTCAGAAAGGTCCGTCAGGTGAACCTGGCAGCCCTGGT
AAAGCAGGTAGTGCCGGTGAGCAGGGTCCGCCGGGCAAAGATGGTAGTAATGGTGA
GCCGGGTAGCCCTGGCAAAGAAGGTGAACGTGGTCTGGCAGGACCGCCGGGTCCTG
ATGGTCGCCGCGGTGAAACGGGTTCACCGGGTATTGCCGGTGCCCTGGGTAAACCA
GGTCTGGAAGGTCCGAAAGGTTATCCTGGTCTGCGCGGTCTGTGATGGTACCAATGGC
AAACGTGGCGAACAGGGCGAAACCGGTCCAGATGGTGTTCGTGGTATTCCGGGTAA
CGATGGTCAGAGCGGTAAACCGGGCATTGATGGTATTGATGGCACCAATGGTCAGC
CTGGCGAAGCAGGTTATCAGGGTGGTCGCGGTACCCGTGGTCAGCTGGGTGAAACA
GGTGATGTTGGTCAGAATGGTGATCGCGGCGCACCGGGTCCGGATGGTAGCAAAGG
TAGCGCCGGTCTGCCGGGTTTACGTAA (SEQ ID NO: 6)

[0142] MKKIWLALAGLVLAFSASAAQYEDGPQGVVGADGKDGTGPNAGQKGPSGEPG
SPGKAGSAGEQGPPGKDGSNGEPGSPGKEGERGLAGPPGPDGRRGETGSPGIAGALGKP
GLEGPKGYPLRGRDGTNGKRGEQGETGPDGVRGIPGNDGQSGKPGIDGIDGTNGQPG
EAGYQGGRGTRGQLGETGDVQGQNGDRGAPGPDGSKGSAGRPGLR (SEQ ID NO: 7)

[0143] A polynucleotide encoding a truncated jellyfish collagen without a His tag, linker and thrombin cleavage site is disclosed in SEQ ID NO: 8.

[0144] GGTCCGCAGGGTGTGTTGGTGCAGATGGTAAAGACGGTACCCCGGGTAATG
CAGGTCAGAAAGGTCCGTCAGGTGAACCTGGCAGCCCTGGTAAAGCAGGTAGTGCC
GGTGAGCAGGGTCCGCCGGGCAAAGATGGTAGTAATGGTGAGCCGGGTAGCCCTGG
CAAAGAAGGTGAACGTGGTCTGGCAGGACCGCCGGGTCTGATGGTCGCCGCGGTG
AAACGGGTTCACCGGGTATTGCCGGTGCCCTGGGTAAACCAGGTCTGGAAGGTCCG
AAAGGTTATCCTGGTCTGCGCGGTCTGTGATGGTACCAATGGCAAACGTGGCGAACA
GGGCGAAACCGGTCCAGATGGTGTTCGTGGTATTCCGGGTAAACGATGGTCAGAGCG
GTAAACCGGGCATTGATGGTATTGATGGCACCAATGGTCAGCCTGGCGAAGCAGGT
TATCAGGGTGGTCGCGGTACCCGTGGTCAGCTGGGTGAAACAGGTGATGTTGGTCA
GAATGGTGATCGCGGCGCACCGGGTCCGGATGGTAGCAAAGGTAGCGCCGGTCGTC
CGGGTTTACGTAA (SEQ ID NO: 8)

[0145] A truncated jellyfish collagen without a His tag, linker, and thrombin cleavage site is disclosed in SEQ ID NO: 5.

[0146] GPQGVVGADGKDGTGPNAGQKGPSGEPGSPGKAGSAGEQGPPGKDGSNGEPGS
PGKEGERGLAGPPGPDGRRGETGSPGIAGALGKPGLEGPKGYPLRGRDGTNGKRGEQ

GETGPDGVRGIPGNDGQSGKPGIDGIDGTNGQPGEAGYQGGRGTRGQLGETGDVVGQNG
DRGAPGPDGSKGSAGRPGLR (SEQ ID NO: 5)

Truncated Collagen with DsbA secretion tag-His tag-Linker-Thrombin cleavage site and GFP Beta-lactamase fusion (version 1):

[0147] A jellyfish collagen with DsbA secretion tag-His tag-Linker-Thrombin cleavage site and GFP Beta-lactamase fusion is disclosed below. The codon-optimized nucleotide sequence encoding this collagen is provided in SEQ ID NO: 9. The amino acid sequence is disclosed in SEQ ID NO: 10. The DsbA secretion tag is encoded by nucleotides 1-72 of SEQ ID NO: 9 and encodes amino acids 1-24 of SEQ ID NO: 10. The His tag is encoded by nucleotides 73-99 of SEQ ID NO: 9 and encodes a 9 histidine tag of amino acids 25-33 of SEQ ID NO: 10. The linker is encoded by nucleotides 100-111 of SEQ ID NO: 9 and encodes amino acids 34-37 of SEQ ID NO: 10. The thrombin cleavage site is encoded by nucleotides 112-135 of SEQ ID NO: 9 and encodes amino acids 38-45 of SEQ ID NO: 10. The green fluorescent protein (GFP) with linker is encoded by nucleotides 136-873 of SEQ ID NO: 9 and encodes amino acids 46-291 of SEQ ID NO: 10. The truncated collagen sequence is encoded by nucleotides 874-1440 of SEQ ID NO: 9 and encodes amino acids 292-480 of SEQ ID NO: 10. The Beta-lactamase with linker is encoded by nucleotides 1441-2232 of SEQ ID NO: 9 and encodes amino acids 481-744 of SEQ ID NO: 10. The Beta-lactamase was properly targeted to the periplasmic space even though the polypeptide did not have an independent secretion tag. The DsbA secretion tag directed the entire transcript (Truncated Collagen with DsbA secretion tag-His tag-Linker-Thrombin cleavage site and GFP Beta-lactamase fusion protein) to the periplasmic space and the Beta-lactamase functioned properly.

[0148] ATGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATC
GGCGGCGCAGTATGAAGATCACCATCACCACCACCATCACCCTCTGGCTCGA
GCCTGGTGCCGCGCGGCAGCCATATGTCTGGCTCGAGCAGTAAAGGTGAAGAACTG
TTCACCGGTGTTGTTCCGATCCTGGTTGAACTGGATGGTGATGTTAACGGCCACAAA
TTCTCTGTTCGTGGTGAAGGTGAAGGTGATGCAACCAACGGTAACTGACCCTGAA
ATTCATCTGCACTACCGGTAACTGCCGGTTCCATGGCCGACTCTGGTGACTACCCT
GACCTATGGTGTTTCAGTGTTTTTCTCGTTACCCGGATCACATGAAGCAGCATGATTTT
TTCAAATCTGCAATGCCGGAAGGTTATGTACAGGAGCGCACCATTTCTTTCAAAGAC
GATGGCACCTACAAAACCCGTGCAGAGGTTAAATTTGAAGGTGATACTCTGGTGAA
CCGTATTGAACTGAAAGGCATTGATTTCAAAGAGGACGGCAACATCCTGGGCCACA
AACTGGAATATAACTTCAACTCCATAACGTTTACATCACCGCAGACAAACAGAAG
AACGGTATCAAAGCTAACTTCAAAATTGCCATAACGTTGAAGACGGTAGCGTACA

GCTGGCGGACCACTACCAGCAGAACAACCTCCGATCGGTGATGGTCCGGTTCTGCTGCC
GGATAACCACTACCTGTCCACCCAGTCTAAACTGTCCAAAGACCCGAACGAAAAGC
GCGACCACATGGTGCTGCTGGAGTTCGTTACTGCAGCAGGTATCACGCACGGCATG
GATGAACTCTACAAATCTGGCGCGCCGGGCGGTCCGCAGGGTGTTGTTGGTGCAGA
TGGTAAAGACGGTACCCCGGGTAATGCAGGTCAGAAAGGTCCGTCAGGTGAACCTG
GCAGCCCTGGTAAAGCAGGTAGTGCCGGTGAGCAGGGTCCGCCGGGCAAAGATGGT
AGTAATGGTGAGCCGGGTAGCCCTGGCAAAGAAGGTGAACGTGGTCTGGCAGGACC
GCCGGGTCTGATGGTCGCCGCGGTGAAACGGGTTCACCGGGTATTGCCGGTGCCCT
GGGTAAACCAGGTCTGGAAGGTCCGAAAGGTTATCCTGGTCTGCGCGGTCTGTGATG
GTACCAATGGCAAACGTGGCGAACAGGGCGAAACCGGTCCAGATGGTGTTCGTGGT
ATTCCGGGTAAACGATGGTCAGAGCGGTAAACCGGGCATTGATGGTATTGATGGCAC
CAATGGTCAGCCTGGCGAAGCAGGTTATCAGGGTGGTCGCGGTACCCGTGGTCAGC
TGGGTGAAACAGGTGATGTTGGTCAGAATGGTGATCGCGGCGCACCGGGTCCGGAT
GGTAGCAAAGGTAGCGCCGGTCGTCCGGGTTACGTCACCCAGAAACGCTGGTGAA
AGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATC
TCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTCCAATGATGA
GCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAG
AGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAG
TCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC
ATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACC
GAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCG
TTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGC
CTGTAGCAATGGCAACAACGTTGCGCAAACCTATTAACCTGGCGAACTACTTACTCTAG
CTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTT
CTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAG
CGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATC
GTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGAT
CGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAA (SEQ ID NO: 9)

[0149] MKKIWLALAGLVLAFSASAAQYEDHHHHHHHHHSGSSLVPRGSHMSGSSSKGE
ELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTCLKFICTTGKLPVPWPTLVTTL
TYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNR
IELKGIDFKEDGNILGHKLEYNFNHSNVYITADKQKNGIKANFKIRHNVEDGSVQLADH
YQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMLLEFVTAAGITHGMDELYKS
GAPGGPQGVVGADGKDGTGPNAGQKGPSGEPGSPGKAGSAGEQGPPEGKDGSNGEPSG

GKEGERGLAGPPGPDGRRGETGSPGIAGALGKPGLEGPKGYPLRGRDGTNGKRGEQG
 ETGPDGVRGIPGNDGQSGKPGIDGIDGTNGQPGEAGYQGGRGTRGQLGETGDVVGQNGD
 RGAPGPDGSKGSAGRPGLRHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERF
 PMMSTFKVLLCGAVLSRIDAGQEQLGRRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCS
 AAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTSLDRWEPELNEAIPNDERDITMP
 VAMATTLRKLLTGELLTLASRQQQLIDWMEADKVAGPLLSALPAGWFIADKSGAGERG
 SRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW (SEQ ID NO: 10)

[0150] The polynucleotide of SEQ ID NO: 9 was constructed by assembling several DNA fragments. The collagen containing sequence was codon optimized and synthesized by Gen9 DNA (now Ginkgo Bioworks) internal synthesis. The GFP was also synthesized by Gen9. The Beta-lactamase was cloned out of the plasmid pKD46

(cgsc2.biology.yale.edu/Strain.php?ID=68099) using PCR with the enzyme PrimeSTAR® GXL polymerase

(www.clontech.com/US/Products/PCR/GC_Rich/PrimeSTAR_GXL_DNA_Polymerase?site=10020:22372:US). Overlaps between the pET28 vector, GFP, Collagen, and Beta-lactamase was designed to be between 30 and 40 bp long and added using PCR with the enzyme PrimeSTAR® GXL polymerase. The opened pET28a vector and inserts were then assembled together into the final plasmid using SGI Gibson Assembly® (us.vwr.com/store/product/17613857/gibson-assembly-hifi-1-step-kit-synthetic-genomics-inc). The plasmid sequence was then verified through Sanger sequencing through Eurofins Genomics (www.eurofinsgenomics.com).

[0151] The transformed cells were cultivated in minimal media and frozen in 1.5 ml aliquots with glycerol at a ratio of 50:50 of cells to glycerol. One vial of this frozen culture was revived in 50 ml of minimal media overnight at 37 °C, 200 rpm. Cells were transferred into 300 ml of minimal media and grown for 6-9 hours to reach an OD600 of 5-10.

[0152] A bioreactor was prepared with 2.7 L of minimal media + glucose and 300 ml of OD600 of 5-10 culture was added to bring the starting volume to 3 L. Cells were grown at 28° C, pH 7 with Dissolved Oxygen maintained at 20% saturation using a cascade containing agitation, air, and oxygen. pH was controlled using 28% w/w ammonium hydroxide solution. Fermentation was run in a fed-batch mode using a DO-stat based feeding algorithm once the initial bolus of 40 g/L was depleted around 13 hours. After 24-26 hours of initial growth, the OD600 reached above 100. At this point, 300 mL of 500 g/L sucrose was added and temperature was reduced to 25° C. High density culture was induced for protein production using 1 mM IPTG. Fermentation was continued for another 20-24 hours and cells were harvested using a bench top centrifuge at 9000 rcf, 15° C for 60 minutes. Cell pellet recovered from centrifugation was resuspended in a buffer

containing 0.5 M NaCl and 0.1 M KH₂PO₄ at pH 8 in a weight by weight ratio of 2x buffer to 1x cells.

[0153] The harvested cells were disrupted in a homogenizer at 14,000 psi pressure in 2 passes. The resulting slurry contained the collagen protein along with other proteins.

[0154] The collagen was purified by acid treatment of non-homogenized whole cells recovered from the bioreactor after centrifugation and resuspension in the buffer described above. The pH of the resuspended suspension was decreased to 3 using 6 M Hydrochloric acid. Acidified cell slurry was incubated overnight at 4° C with mixing, followed by centrifugation. The pH was then raised to 9 using 10 N NaOH and the supernatant of the slurry was tested on a polyacrylamide gel and found to contain collagen in relatively high abundance compared to starting pellet. The collagen slurry thus obtained was high in salts. To obtain volume and salt reduction, concentration and diafiltration steps were performed using an EMD Millipore Tangential Flow Filtration system with ultrafiltration cassettes of 0.1 m² each. Total area of filtration was 0.2 m² using 2 cassettes in parallel. A volume reduction of 5x and a salt reduction of 19x was achieved in the TFF stage. Final collagen slurry was run on an SDS-PAGE gel to confirm presence of the collagen. This slurry was dried using a multi-tray lyophilizer over 3 days to obtain a white, fluffy collagen powder.

[0155] The purified collagen-GFP-Beta-lactamase fusion protein was analyzed on an SDS-PAGE gel and was observed to run at an apparent molecular weight of 90 kilodaltons. The expected size of the fusion protein is 85 kDa. The 90 kDa band was confirmed by mass spectrometry to be the correct collagen fusion protein.

Truncated Collagen with DsbA secretion tag-His tag-Linker-Thrombin cleavage site and GFP Beta-lactamase fusion (version 2):

[0156] A jellyfish collagen with DsbA secretion tag-His tag-Linker-Thrombin cleavage site and GFP Beta-lactamase fusion is disclosed below. The codon-optimized nucleotide sequence encoding this collagen is provided in SEQ ID NO: 11. The amino acid sequence is disclosed in SEQ ID NO: 12. The DsbA secretion tag is encoded by nucleotides 1-72 of SEQ ID NO: 11 and encodes amino acids 1-24 of SEQ ID NO: 12. The His tag is encoded by nucleotides 73-99 of SEQ ID NO: 11 and encodes a 9 histidine tag of amino acids 25-33 of SEQ ID NO: 12. The linker is encoded by nucleotides 100-111 of SEQ ID NO: 11 and encodes amino acids 34-37 of SEQ ID NO: 12. The thrombin cleavage site is encoded by nucleotides 112-135 of SEQ ID NO: 11 and encodes amino acids 38-45 of SEQ ID NO: 12. The green fluorescent protein (GFP) with linker is encoded by nucleotides 136-873 of SEQ ID NO: 11 and encodes amino acids 46-291 of SEQ ID NO: 12. The truncated collagen sequence is encoded by nucleotides 874-1440 of SEQ

ID NO: 11 and encodes amino acids 292-480 of SEQ ID NO: 12. The Beta-lactamase with linker is encoded by nucleotides 1441-2232 of SEQ ID NO: 11 and encodes amino acids 481-744 of SEQ ID NO: 12.

[0157] ATGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATC
GGCGGCGCAGTATGAAGATCACCATCACCACCACCACCATCACCCTCTGGCTCGA
GCCTGGTGCCGCGCGGCAGCCATATGTCTGGCTCGAGCAGTAAAGGTGAAGAACTG
TTCACCGGTGTTGTTCCGATCCTGGTTGAACTGGATGGTGATGTTAACGGCCACAAA
TTCTCTGTTTCGTGGTGAAGGTGAAGGTGATGCAACCAACGGTAACTGACCCTGAA
ATTCATCTGCACTACCGGTAACTGCCGGTTCATGGCCGACTCTGGTGACTACCCT
GACCTATGGTGTTCAAGTGTTCCTCTCGTTACCCGGATCACATGAAGCAGCATGATTC
TTCAAATCTGCAATGCCGGAAGGTTATGTACAGGAGCGCACCATTTCTTTCAAAGAC
GATGGCACCTACAAAACCCGTGCAGAGGTTAAATTTGAAGGTGATACTCTGGTGAA
CCGTATTGAACTGAAAGGCATTGATTTCAAAGAGGACGGCAACATCCTGGGCCACA
AACTGGAATATAACTTCAACTCCCATACGTTTACATCACCGCAGACAAACAGAAG
AACGGTATCAAAGCTAACTTCAAAAATTCGCCATAACGTTGAAGACGGTAGCGTACA
GCTGGCGGACCACTACCAGCAGAACACTCCGATCGGTGATGGTCCGGTTCCTGCTGCC
GGATAACCACTACCTGTCCACCCAGTCT^{Taa}CTGTCCAAAGACCCGAACGAAAAGCG
CGACCACATGGTGCTGCTGGAGTTCGTTACTGCAGCAGGTATCACGCACGGCATGG
ATGAACTCTACAAATCTGGCGCGCCGGGCGGTCCGCAGGGTGTGTTGGTGCAAGAT
GGTAAAGACGGTACCCCGGGTAATGCAGGTCAGAAAGGTCCGTCAGGTGAACCTGG
CAGCCCTGGTAAAGCAGGTAGTGCCGGTGAGCAGGGTCCGCCGGGCAAAGATGGTA
GTAATGGTGAGCCGGGTAGCCCTGGCAAAGAAGGTGAACGTGGTCTGGCAGGACCG
CCGGGTCCTGATGGTCGCCGCGGTGAAACGGGTTCACCGGGTATTGCCGGTGCCCTG
GGTAAACAGGTCTGGAAGGTCCGAAAGGTTATCCTGGTCTGCGCGGTCTGTATGG
TACCAATGGCAAACGTGGCGAACAGGGCGAAACCGGTCCAGATGGTGTTCTGTGGTA
TTCCGGGTAACGATGGTCAGAGCGGTAAACCGGGCATTGATGGTATTGATGGCACC
AATGGTCAGCCTGGCGAAGCAGGTTATCAGGGTGGTCGCGGTACCCGTGGTCAGCT
GGGTGAAACAGGTGATGTTGGTCAGAATGGTGATCGCGGCGCACCGGGTCCGGATG
GTAGCAAAGGTAGCGCCGGTCTGCCGGGTTTACGTACCCAGAAACGCTGGTGAAA
GTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCT
CAACAGCGGTAAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTCCAATGATGAG
CACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGA
GCAACTCGGTGCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTACCAAGT
CACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCA

TAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCG
 AAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGT
 TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCC
 TGTAGCAATGGCAACAACGTTGCGCAAACCTATTAACCTGGCGAACTACTTACTCTAGC
 TTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTC
 TGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGC
 GTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCG
 TAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATC
 GCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAA (SEQ ID NO: 11)

[0158] MKKIWLALAGLVLAFTSASAAQYEDHHHHHHHHHSGSSLVPRGSHMSGSSSKGE
 ELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTCLKFICTTGKLPVPWPTLVTTL
 TYGVQCFSRYPDHMKQHDFKFSAMPEGYVQERTISFKDDGTYSKTRAEVKFEGDTLVNR
 IELKGIDFKEDGNILGHKLEYNFSHNHVVITADKQKNGIKANFKIRHNVEDGSVQLADH
 YQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMLLEFVTAAGITHGMDLEYKS
 GAPGGPQGVVGADGKDGTGPNAGQKGPSGEPGSPGKAGSAGEQGPPGKDGSGNGEPGSP
 GKEGERGLAGPPGPDGRRGETGSPGIAGALGKPGLEGPKGYPLRGRDGTNGKRGEQG
 ETGPDGVRGIPGNDGQSGKPGIDGIDGTNGQPGEAGYQGGRGTRGQLGETGDVVGQNGD
 RGAPGPDGSKGSAGRPLRHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERF
 PMMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCS
 AAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTSLDRWEPELNEAIPNDERDITMP
 VAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSLPAGWFIADKSGAGERG
 SRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW (SEQ ID NO: 12)

Example 2. Human Collagens

Truncated Human Collagen Type 21 alpha 1

[0159] A truncated human collagen type 21 alpha 1 (truncated relative to full-length human type 21 alpha 1 collagen (SEQ ID NO: 31)) without a His tag, linker, and thrombin cleavage site is disclosed below. The codon-optimized nucleotide sequence encoding this collagen and the amino acid sequence are disclosed below. The DsbA secretion tag is encoded by nucleotides 1-72 of SEQ ID NO: 13 and encodes amino acids 1-24 of SEQ ID NO: 14. The truncated collagen sequence is encoded by nucleotides 73-633 of SEQ ID NO: 13 and encodes amino acids 25-211 of SEQ ID NO: 14.

[0160] The codon-optimized nucleotide sequence encoding this collagen is provided in SEQ ID NO: 13.

[0161] ATGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATC
GGCGGCGCAGTATGAAGATGCAGGTTTTCCGGGTCTGCCTGGTCCGGCAGGCGAAC
CGGGTCGTCATGGTAAAGATGGTCTGATGGGTAGTCCGGGTTTTAAAGGTGAAGCA
GGTTCACCGGGTGCACCTGGTCAGGATGGCACCCGTGGTGAACCGGGTATTCCGGG
ATTTCCGGGTAATCGTGGCCTGATGGGTGAGAAAGGTGAAATTGGTCCGCCTGGTCA
GCAGGGTAAAAAAGGCGCACCGGGTATGCCAGGACTGATGGGTTCAAATGGCAGTC
CGGGTCAGCCAGGCACACCGGGTTCAAAGGTAGCAAAGGCGAACCTGGTATTTCAG
GGTATGCCTGGTGCAAGCGGTCTGAAAGGCGAGCCAGGTGCCACCGGTTCTCCGGG
TGAACCAGGTTATATGGGTCTGCCAGGTATCCAAGGCAAAAAAGGTGATAAAGGTA
ATCAGGGCGAAAAAGGCATTTCAGGGCCAGAAAGGCGAAAAATGGCCGTCAGGGTAT
TCCAGGCCAGCAGGGCATCCAGGGTCATCATGGTGCAAAAGGTGAACGTGGTGAAA
AGGGCGAACCAAGGTGTTTCGTTTA (SEQ ID NO: 13)

[0162] The amino acid sequence is disclosed in SEQ ID NO: 14.

[0163] MKKIWLALAGLVLAFLSASAAQYEDAGFPGLPGPAGEPGRHGKDGLMGSPGFKG
EAGSPGAPGQDGRGEPGIPGFPGNRGLMGQKGEIGPPGQQGKKGAPGMPGLMGSNGS
PGQPGTPGSKGSKGEPGIQGMPPGASGLKGEPGATGSPGEPGYMGLPGIQGKKGDKGNQ
GEKGIQGGQKGENGRQGIPGQQGIQGHGAKGERGEKGEPPGVR (SEQ ID NO: 14)

[0164] The codon-optimized nucleotide sequence encoding the truncated human collagen type 21 alpha 1 without the DsbA secretion tag collagen is provided in SEQ ID NO: 15.

[0165] TGCAGGTTTTCCGGGTCTGCCTGGTCCGGCAGGCGAACCGGGTCGTCATGGT
AAAGATGGTCTGATGGGTAGTCCGGGTTTTAAAGGTGAAGCAGGTTACCGGGTGC
ACCTGGTCAGGATGGCACCCGTGGTGAACCGGGTATTCCGGGATTTCCGGGTAATCG
TGGCCTGATGGGTGAGAAAGGTGAAATTGGTCCGCCTGGTCAGCAGGGTAAAAAAG
GCGCACCGGGTATGCCAGGACTGATGGGTTCAAATGGCAGTCCGGGTGTCAGCCAGGC
ACACCGGGTTCAAAGGTAGCAAAGGCGAACCTGGTATTTCAGGGTATGCCTGGTGC
AAGCGGTCTGAAAGGCGAGCCAGGTGCCACCGGTTCTCCGGGTGAACCAGGTTATA
TGGGTCTGCCAGGTATCCAAGGCAAAAAAGGTGATAAAGGTAATCAGGGCGAAAA
AGGCATTTCAGGGCCAGAAAGGCGAAAAATGGCCGTCAGGGTATTCCAGGCCAGCAGG
GCATCCAGGGTCATCATGGTGCAAAAGGTGAACGTGGTGAAAAGGGCGAACCAAGGT
GTTTCGTTAA (SEQ ID NO: 15)

[0166] The amino acid sequence of truncated human collagen type 21 alpha 1 without the DsbA secretion tag is disclosed in SEQ ID NO: 16.

[0167] AGFPGLPGPAGEPGRHGKDGLMGSPGFKGEAGSPGAPGQDGRGEPGIPGFPGN
RGLMGQKGEIGPPGQQGKKGAPGMPGLMGSNGSPGQPGTPGSKGSKGEPGIQGMPPGAS

GLKGEPGATGSPGEPGYMGLPGIQGKKGDKGNQGEKGIQGQKGNGRQGIPGQQGIQG
HHGAKGERGEKGEPGVR (SEQ ID NO: 16)

[0168] The polynucleotides of SEQ ID NO: 13 were synthesized by Twist Bioscience. Overlaps between the pET28 vector and SEQ ID NO: 15 and SEQ ID NO: 16 were designed to be between 20 and 30 bp long and added using PCR with the enzyme PrimeSTAR® GXL polymerase (www.takarabio.com/products/pcr/gc-rich-pcr/primestar-gxl-dna-polymerase). The opened pET28a vector and insert DNA (SEQ ID NO: 13) was then assembled together into the final plasmid using In-Fusion Cloning (www.takarabio.com/products/cloning/in-fusion-cloning). The plasmid sequence was then verified through Sanger sequencing through Genewiz (www.genewiz.com/en).

[0169] The transformed cells were cultivated in minimal media and frozen in 1.5 ml aliquots with vegetable glycerin at a ratio of 50:50 of cells to glycerin. One vial of this frozen culture was revived in 50 ml of minimal media overnight at 37° C, 200 rpm. Cells were transferred into 300 ml of minimal media and grown for 6-9 hours to reach an OD600 of 5-10.

[0170] Minimal media used in this example and throughout this application is prepared as follows:

1) Autoclave 5 L of 550 g/kg Glucose syrup at concentration in DI water. (VWR, product #97061-170).

2) Autoclave in 3946 mL of DI water:

20 g (NH₄)₂HPO₄. (VWR, product # 97061-932);

66.5 g KH₂PO₄. (VWR, product # 97062-348);

22.5 g H₃C₆H₅O₇. (VWR, product #BDH9228-2.5KG);

8.85 g MgSO₄·7H₂O. (VWR, product # 97062-134);

10 mL of 1000x Trace metals formulation (Table 3).

After autoclaving, add

118 g of (1) to (2);

5 mL of 25 mg/mL Kanamycin Sulfate (VWR-V0408);

Use 28% NH₄OH (VWR, product #BDH3022) to adjust pH to 6.1.

Table 3. Trace metals formulation

Ferrous Sulfate Heptahydrate, 27.8 g/L (Spectrum, 7782-63-0)
Zinc Sulfate heptahydrate, 2.88 g/L (Spectrum, 7446-20-0)
Calcium chloride dihydrate, 2.94 g/L (Spectrum, 2971347)
Sodium molybdate dihydrate, 0.48 g/L (Spectrum, 10102-40-6)

Manganese chloride tetrahydrate, 1.26 g/L (Spectrum, 13446-34-9)
Sodium selenite, 0.35 g/L (Spectrum, 10102-18-8)
Boric acid, 0.12 g/L (Spectrum, 10043-35-3)

[0171] The fermentations were performed at various temperature ranging from 25° to 28° C. For some fermentations, the temperature of the fermentation was maintained at a constant temperature and immediately upon completion of fermentation the collagen was purified. For other fermentations, the temperature of the fermentations was maintained for a desired period of time and when cell densities of OD600 of 10-20 were reached, the temperature was reduced to induce protein production. Typically, the temperature was reduced from 28° C to 25° C. After the fermentation at 25° C was continued for 40-60 hours.

[0172] The collagen was purified as follows: The pH of the fermentation broth was decreased to between 3-3.5 using 5-50% Sulfuric Acid. The cells were then separated using centrifugation. Supernatant of the acidified broth was tested on a polyacrylamide gel and found to contain collagen in relatively high abundance compared to starting pellet. The collagen slurry thus obtained was high in salts. To obtain volume and salt reduction, concentration and diafiltration steps were performed using an EMD Millipore Tangential Flow Filtration system with ultrafiltration cassettes of 0.1 m2 each. Total area of filtration was 0.2 m2 using 2 cassettes in parallel. A volume reduction of 5x and a salt reduction of 19x was achieved in the TFF stage. Final collagen slurry was run on an SDS-PAGE gel to confirm presence of the collagen.

[0173] The purified collagen was analyzed on an SDS-PAGE gel and a thick and clear band was observed at the expected size of 25 kilodaltons. Quantification of collagen titers and purity were conducted using reverse phase and size exclusion HPLC chromatography. Titters are usually between 3 to 8 grams per liter. The purified collagen was also further analyzed by mass spectrometry and it was confirmed to match the published sequence of human type 21 collagen.

Truncated Human Collagen type 1 alpha 2 (1)

[0174] A truncated human collagen type 1 alpha 2 (truncated relative to full-length human collagen type 1 alpha 2 (SEQ ID NO: 32)) without a His tag, linker, and thrombin cleavage site is disclosed below. The codon-optimized nucleotide sequence and the amino acid sequences are disclosed below. The DsbA secretion tag is encoded by nucleotides 1-72 of SEQ ID NO: 17 and encodes amino acids 1-24 of SEQ ID NO: 18. The truncated collagen sequence is encoded by nucleotides 73-636 of SEQ ID NO: 17 and encodes amino acids 25-212 of SEQ ID NO: 18.

[0175] The codon-optimized nucleotide sequence encoding this collagen is provided in SEQ ID NO: 17.

[0176] ATGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATC
GGCGGCGCAGTATGAAGATATGGGTCCGCCTGGTAGCCGTGGTGCAAGTGGTCCGG
CAGGCGTTCGTGGTCCGAATGGTGATGCAGGTCGTCCGGGTGAACCGGGTCTGATG
GGTCCTCGTGGTCTGCCTGGTTCACCGGGTAATATTGGTCCTGCAGGTAAAGAAGGT
CCGGTTGGTCTGCCAGGTATTGATGGCCGTCCGGGTCCGATTGGTCCAGCCGGTGCA
CGTGGTGAACCTGGCAATATTGGTTTTCCGGGTCTCTAAAGGTCCGACCGGTGATCCG
GGTAAAAATGGTGATAAAGGTCATGCAGGTCTGGCAGGCGCACGCGGTGCACCTGG
TCCGGATGGTAATAATGGTGACAGGGTCCACCGGGTCCGCAGGGTGTTCAAGGTG
GTAAAGGCGAACAGGGTCTGCCGGTCTCCGGGTTTTTCAGGGACTGCCTGGTCCGA
GCGGTCTGCGGGTGAAGTTGGTAAACCTGGTGAACGCGGTCTGCATGGTGAATTTG
GCCTGCCTGGGCCTGCAGGTCCGCGTGGCGAACGTGGTCCGCCAGGTGAAAGCGGT
GCAGCAGGTCCGACAGGTAA (SEQ ID NO: 17)

[0177] The amino acid sequence is disclosed in SEQ ID NO: 18.

[0178] MKKIWLALAGLVLAFSASAAQYEDMGPPGSRGASGPAGVRGPNGDAGRPGEPG
LMGPRGLPGSPGNIGPAGKEGPVGLPGIDGRPGPIGPAGARGEPPGNIGFPGPKGPTGDPG
KNGDKGHAGLAGARGAPGPDGNNGAQGPPGPQGVQGGKGEQGPAGPPGFQGLPGPSG
PAGEVGKPGERGLHGEFGLPGPAGPRGERGPPGESGAAGPTG (SEQ ID NO: 18)

[0179] The nucleic acid sequence of truncated human collagen type 1 alpha 2(1) without the
DsbA secretion tag is disclosed in SEQ ID NO: 19.

[0180] ATGGGTCCGCCTGGTAGCCGTGGTGCAAGTGGTCCGGCAGGCGTTCGTGGTC
CGAATGGTGATGCAGGTCGTCCGGGTGAACCGGGTCTGATGGGTCTCTCGTGGTCTGC
CTGGTTCACCGGGTAATATTGGTCTCTGCAGGTAAAGAAGGTCCGGTTGGTCTGCCAG
GTATTGATGGCCGTCCGGGTCCGATTGGTCCAGCCGGTGACGTGGTGAACCTGGCA
ATATTGGTTTTCCGGGTCTCTAAAGGTCCGACCGGTGATCCGGGTAAAAATGGTGATA
AAGGTCATGCAGGTCTGGCAGGCGCACGCGGTGCACCTGGTCCGGATGGTAATAAT
GGTGCACAGGGTCCACCGGGTCCGCAGGGTGTTCAAGGTGGTAAAGGCGAACAGGG
TCCTGCCGGTCTCCGGGTTTTTCAGGGACTGCCTGGTCCGAGCGGTCTGCGGGTGA
AGTTGGTAAACCTGGTGAACGCGGTCTGCATGGTGAATTTGGCCTGCCTGGGCCTGC
AGGTCCGCGTGGCGAACGTGGTCCGCCAGGTGAAAGCGGTGCAGCAGGTCCGACAG
GTAA (SEQ ID NO: 19)

[0181] The amino acid sequence of truncated human collagen type 1 alpha 2(1) without the
DsbA secretion tag is disclosed in SEQ ID NO: 20.

[0182] MGPPGSRGASGPAGVRGPNGDAGRPGEPGLMGPRGLPGSPGNIGPAGKEGPVGL
PGIDGRPGPIGPAGARGEPPGNIGFPGPKGPTGDPGKNGDKGHAGLAGARGAPGPDGNNG

AQGPPGPQGVQGGKGEQGPAGPPGFQGLPGPSGPAGEVGKPGERGLHGEFGLPGPAGP
RGERGPPGESGAAGPTG (SEQ ID NO: 20)

Truncated Human Collagen type 1 alpha 2 (2)

[0183] A truncated human collagen type 1 alpha 2 (truncated relative to full-length human collagen type 1 alpha 2 (SEQ ID NO: 32)) without a His tag, linker, and thrombin cleavage site is disclosed below. The codon-optimized nucleotide sequence and the amino acid sequences are disclosed below. The DsbA secretion tag is encoded by nucleotides 1-72 of SEQ ID NO: 21 and encodes amino acids 1-24 of SEQ ID NO: 22. The truncated collagen sequence is encoded by nucleotides 73-609 of SEQ ID NO: 21 and encodes amino acids 25-203 of SEQ ID NO: 22.

[0184] The codon-optimized nucleotide sequence encoding this collagen is provided in SEQ ID NO: 21.

[0185] ATGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATC
GGCGGCGCAGTATGAAGATGGTTTTTCAGGGTCCTGCCGGTGAACCGGGTGAACCTG
GTCAGACAGGTCCGGCAGGCGCACGTGGTCCTGCAGGTCCTCCTGGTAAAGCCGGT
GAAGATGGTCATCCGGGTAAACCGGGTCGTCCTGGTGAACGTGGTGTTGTTGGTCCG
CAGGGTGCCCGTGGTTTTCCGGGTACTCCGGGTCTGCCAGGTTTTAAAGGTATTTCG
GGTCATAATGGTCTGGATGGTCTGAAAGGTCAGCCTGGTGCACCGGGTGTTAAAGG
TGAACCAGGTGCTCCGGGTGAAAATGGCACACCGGGTCAGACCGGTGCGCGTGGTC
TGCCTGGCGAACGCGGTCTGTGTTGGTGCACCTGGTCCAGCCGGTGCACGCGGTAGTG
ATGGTAGCGTTGGTCCGGTTGGTCCAGCGGGTCCGATTGGTAGCGCAGGTCCACCGG
GTTTTCCAGGCGCACCGGGTCCGAAAGGTGAAATTGGTGCAGTTGGTAATGCAGGC
CCTGCCGGTCCAGCAGGACCGCGTGGTGAAGTTGGCCTGCCTGGTCTGTAA (SEQ ID
NO: 21)

[0186] The amino acid sequence is disclosed in SEQ ID NO: 22.

[0187] MKKIWLALAGLVLAFSASAAQYEDGFQGPAGEPGEPGQTGPAGARGPAGPPGK
AGEDGHPGKPRPGERGVVGPQGARGFPPTPLPGFKGIRGHNGLDGLKGQPGAPGVK
GEPGAPGENGTPGQTGARGLPGERGRVGAPGPAGARGSDGSVGPVGPAGPIGSAGPPGF
PGAPGPKGEIGAVGNAGPAGPAGPRGEVGLPGL (SEQ ID NO: 22)

[0188] The nucleic acid sequence of truncated human collagen type 1 alpha 2(2) without the DsbA secretion tag is disclosed in SEQ ID NO: 23.

[0189] GGTTTTTCAGGGTCCTGCCGGTGAACCGGGTGAACCTGGTCAGACAGGTCCGG
CAGGCGCACGTGGTCCTGCAGGTCCTCCTGGTAAAGCCGGTGAAGATGGTCATCCG
GGTAAACCGGGTCGTCCTGGTGAACGTGGTGTTGTTGGTCCGCAGGGTGCCCGTGGT
TTTCCGGGTACTCCGGGTCTGCCAGGTTTTAAAGGTATTTCGTGGTCATAATGGTCTG

GATGGTCTGAAAGGTCAGCCTGGTGCACCGGGTGTTAAAGGTGAACCAGGTGCTCC
GGGTGAAAATGGCACACCGGGTCAGACCGGTGCGCGTGGTCTGCCTGGCGAACGCG
GTCGTGTTGGTGCACCTGGTCCAGCCGGTGCACGCGGTAGTGATGGTAGCGTTGGTC
CGGTTGGTCCAGCGGGTCCGATTGGTAGCGCAGGTCCACCGGGTTTTCCAGGCGCAC
CGGGTCCGAAAGGTGAAATTGGTGCAGTTGGTAATGCAGGCCCTGCCGGTCCAGCA
GGACCGCGTGGTGAAGTTGGCCTGCCTGGTCTGTAA (SEQ ID NO: 23)

[0190] The amino acid sequence of truncated human collagen type 1 alpha 2(2) without the DsbA secretion tag is disclosed in SEQ ID NO: 24.

[0191] GFQGPAGEPGEPGQTGPAGARGPAGPPGKAGEDGHPGKPRPGERGVVGPQGA
RGFPPTPLPGFKGIRGHNGLDGLKGQPGAPGVKGEPGAPGENGTPGQTGARGLPGER
GRVGAPGPAGARGSDGSVGPVGPAGPIGSAGPPGFPAPGPKGEIGAVGNAGPAGPAGP
RGEVGLPGL (SEQ ID NO: 24)

[0192] The polynucleotides of SEQ ID NO: 13, 17, or 21 were subcloned in vector pET28a as described herein to prepare a transformation vector. Host cells were transformed with the vector the polynucleotides were expressed as described in Example 1.

[0193] After the fermentation was completed, the truncated human collagen was purified from the fermentation broth using the procedures disclosed in Example 2. The purified truncated human collagens were analyzed using SDS-PAGE and HPLC as disclosed in Example 2.

[0194] All three truncated human collagens ran at the expected molecular weights in the SDS-PAGE analysis. In analyzing the truncated human collagens using HPLC, a standard curve using the jellyfish collagen of Example 1 was utilized. The retention times of the human collagens were slightly different than the jellyfish collagen. The retention time of SEQ ID NO: 16 was 5.645 minutes, the retention time of SEQ ID NO: 20 was 5.631 minutes, and SEQ ID NO: 24 ran at two peaks and the retention times were 5.531 and 5.7 minutes.

Truncated Human Collagen Type 1 alpha 2 truncation 5 with DsbA secretion and FLAG tag

[0195] The amino acid sequence of truncated human collagen type 1 alpha 2 truncation 5 with DsbA secretion and FLAG tag is disclosed in SEQ ID NO: 25. The DsbA secretion tag is encoded by nucleotides 1-57 of SEQ ID NO: 26 and the amino acid sequences are amino acids 1-19 of SEQ ID NO: 25. The collagen nucleotide sequences are nucleotides 58-657 of SEQ ID NO: 26 and the amino acid sequences are amino acids 20-219 of SEQ ID NO: 25. The FLAG nucleotide sequences are nucleotides 658-684 of SEQ ID NO: 26 and the amino acid sequences are amino acids 220-228 of SEQ ID NO: 25.

[0196] MKKIWLALAGLVLAFSASAGDQGPVGRGTGEVGA VGPPGFAGEKGPSGEAGTAG
PPGTPGPQGLLGAPGILGLPGSRGERGLPGVAGAVGEPGLGIAGPPGARGPPGAVGSPG
VNGAPGEAGRDGNPGNDGPPGRDGQPGHKGERGYPGNIGPVGAAGAPGPHGPVGPAG
KHGNRGETGPSGPVGPAGAVGPRGPSGPQGI RGDKGEPGEKGPRGLPGLGDYKDDDDK
(SEQ ID NO: 25)

[0197] The nucleic acid sequence of truncated human collagen type 1 alpha 2 truncation 5 with DsbA secretion and FLAG tag is disclosed in SEQ ID NO: 26.

[0198] ATGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATC
GGCGGGTGATCAGGGTCCGGTTGGTTCGTACCGGTGAAGTTGGTGCAGTCGGGCCCGC
CGGGTTTTGCGGGTGAAAAAGGCCCGTCAGGTGAAGCAGGCACCGCTGGCCCTCCT
GGCACGCCTGGCCACAGGGTTTACTGGGCGCACCTGGAATTCTGGGACTGCCGGG
CAGCCGTGGAGAACGCGGTTTACCAGGTGTTGCCGGTGCCGTTGGTGAACCTGGTCC
ACTGGGCATTGCAGGGCCGCCTGGCGCACGGGGACCGCCTGGTGCTGTTGGTAGTC
CGGGTGTGAATGGTGCTCCGGGTGAAGCCGGTCGTGACGGTAATCCGGGAAATGAC
GGCCCGCCAGGCCGCGATGGTCAGCCGGGTCATAAAGGTGAGCGTGGTTACCCAGG
TAATATTGGTCCAGTCGGTGCCGCCGGTGCGCCGGGTCTCATGGCCCTGTCGGTCC
AGCCGGTAAACATGGTAATCGCGGTGAGACAGGTCCGTCAGGACCAGTGGGCCCTG
CTGGCGCAGTCGGTCCGCGCGGGCCGAGTGGCCCTCAGGGTATTCGTGGCGATAAA
GGGGAACCGGGCGAAAAAGGGCCGCGGGGTCTGCCAGGCCTGGGTGACTACAAAG
ACGACGACGACAAATAA (SEQ ID NO: 26)

[0199] The polynucleotide of SEQ ID NO: 26 was subcloned into vector pET28a, expressed in host E. coli cells and the truncated collagen was purified as described herein. The purified collagen produced a clear band on SDS-PAGE and an anti-FLAG western was observed at around 100 kilodaltons. There were no existing bands that appear at that location on the gel in the absence of expression of this protein.

Truncated Human Collagen Type 1 alpha 2 truncation 6 with DsbA secretion and FLAG tag

[0200] The amino acid sequence of truncated human collagen type 1 alpha 2 truncation 6 with DsbA secretion and FLAG tag is disclosed in SEQ ID NO: 27. The DsbA secretion tag is encoded by nucleotides 1-57 of SEQ ID NO: 28 and the amino acid sequences are amino acids 1-19 of SEQ ID NO: 27. The collagen nucleotide sequences are nucleotides 58-657 of SEQ ID NO: 28 and the amino acid sequences are amino acids 20-219 of SEQ ID NO: 27. The FLAG nucleotide sequences are nucleotides 658-684 of SEQ ID NO: 28 and the amino acid sequences are amino acids 220-228 of SEQ ID NO: 27.

[0201] MKKIWLALAGLVLAFSASAKGHNLQGLPGIAGHHGDQGAPGSVGPAGPRGPA
GPSGPAGKDGRTGHPGTVGPAGIRGPQGHQGPAGPPGPPGPPGPPGVSGGGYDFGYDG
DFYRADQPRAPSRLPKDYEVDATLKSLNNQIETLLTPEGSRKNPARTCRDLRLSHPEWS
SGYYWIDPNQGCTMDAIKVYCDFSTGETCIRAQPENIPAKNWYRSSKDGDYKDDDDK
(SEQ ID NO: 27)

[0202] The nucleic acid sequence of truncated human collagen type 1 alpha 2 truncation 6 with DsbA secretion and FLAG tag is disclosed in SEQ ID NO: 28.

[0203] ATGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATC
GGCGAAAGGTCACAATGGACTGCAAGGCCTGCCAGGTATTGCAGGTCATCATGGTG
ATCAAGGTGCCCCGGGAAGCGTTGGTCCGGCGGGGCCGAGAGGCCCTGCGGGACCT
TCAGGTCCGGCAGGCAAAGATGGTCGGACAGGCCATCCGGGCACCGTTGGCCCTGC
AGGAATTTCGTGGACCGCAGGGTCATCAGGGACCTGCTGGTCCGCCAGGTCCCCCGG
GCCCTCCGGGACCACCGGGTGTAGTGGTGGTGGTTATGATTTTGGCTATGATGGTG
ATTTTTATCGTGCAGATCAGCCGCGTAGCGCACCGAGCCTGCGTCCTAAAGATTATG
AAGTTGATGCAACCCTGAAAAGCCTGAATAATCAGATTGAAACACTGCTGACACCG
GAAGGTAGCCGTAAAAATCCGGCCCCGTACCTGTCTGTGATCTGCGTCTGAGCCACCCG
GAATGGAGCAGCGGTTATTATTGGATTGATCCGAATCAAGGTTGTACCATGGATGCA
ATTAAAGTTTATTGTGATTTTAGCACAGGTGAAACATGTATCCGTGCACAGCCGGAA
AATATTCCGGCCAAAAATTGGTATCGTAGTAGCAAAGATGGTGACTACAAAGACGA
CGACGACAAATAA (SEQ ID NO: 28)

[0204] The polynucleotide of SEQ ID NO: 28 was subcloned into vector pET28a, expressed in host E. coli cells and the truncated collagen was purified as described herein. The purified collagen produced a clear band on SDS-PAGE and an anti-FLAG western was observed at around 25 kilodaltons. There were no existing bands that appear at that location on the gel in the absence of expression of this protein.

Truncated Human Collagen Type 1 alpha 2 truncation 7 with DsbA secretion and FLAG tag

[0205] The amino acid sequence of truncated human collagen type 1 alpha 2 truncation 7 with DsbA secretion and FLAG tag is disclosed in SEQ ID NO: 29. The DsbA secretion tag is encoded by nucleotides 1-57 of SEQ ID NO: 30 and the amino acid sequences are amino acids 1-19 of SEQ ID NO: 29. The collagen nucleotide sequences are nucleotides 58-759 of SEQ ID NO: 30 and the amino acid sequences are amino acids 20-253 of SEQ ID NO: 29. The FLAG nucleotide sequences are nucleotides 760-786 of SEQ ID NO: 30 and the amino acid sequences are amino acids 254-262 of SEQ ID NO: 29.

[0206] MKKIWLALAGLVLAFSASAYEVDATLKSNNQIETLLTPEGSRKNPARTCRDLR
LSHPEWSSGYWIDPNQGCTMDAIKVYCDFSTGETCIRAQPENIPAKNWYRSSKDKKH
VWLGETINAGSQFEYNVEGVTSKEMATQLAFMRLLANYASQNITYHCKNSIAYMDEET
GNLKKAVILQGSNDVELVAEGNSRFTYTVLVDGCSKKTNEWGKTIIEYKTNKPSRLPFL
DIAPLDIGGADQEFFVDIGPVCFKGDYKDDDDK (SEQ ID NO: 29)

[0207] The nucleic acid sequence of truncated human collagen type 1 alpha 2 truncation 7 with DsbA secretion and FLAG tag is disclosed in SEQ ID NO: 30.

[0208] TGAAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATCG
GCGTATGAAGTTGATGCAACCCTGAAAAGCCTGAATAATCAGATTGAAACACTGCT
GACACCGGAAGGTAGCCGTAAAAATCCGGCCCGTACCTGTCGTGATCTGCGTCTGA
GCCACCCGGAATGGAGCAGCGGTTATTATTGGATTGATCCGAATCAAGGTTGTACCA
TGGATGCAATTAAGTTTATTGTGATTTTAGCACAGGTGAAACATGTATCCGTGCAC
AGCCGGAAAATATTCCGGCCAAAAATTGGTATCGTAGTAGCAAAGATAAAAAACAT
GTGTGGCTGGGTGAAACCATTAATGCAGGTAGCCAGTTTGAATACAATGTTGAAGG
TGTTACCAGCAAAGAAATGGCAACACAGCTGGCATTATGCGTCTGCTGGCAAATTA
TGCAAGCCAGAATATTACATATCATTGTAAAAATAGCATTGCATATATGGATGAAG
AAACCGGTAATCTGAAAAAAGCAGTTATTCTGCAGGGTAGCAATGATGTTGAACTG
GTTGCCGAAGGTAATAGCCGTTTTACATATACCGTTCTGGTTGATGGTTGTAGCAAA
AAAACCAATGAATGGGGTAAAACCATCATTGAATATAAAACCAACAAACCGAGCCG
TCTGCCGTTTCTGGATATCGCTCCGCTGGATATTGGTGGTGCCGATCAGGAATTTTTT
GTCGATATCGGTCCTGTGTGTTTTAAAGGTGACTACAAAGACGACGACGACAAATA
A (SEQ ID NO: 30)

[0209] The polynucleotide of SEQ ID NO: 30 was subcloned into vector pET28a, expressed in host E. coli cells and the truncated collagen was purified as described herein. The purified collagen produced a clear band on SDS-PAGE and an anti-FLAG western was observed at around 30 kilodaltons. There were no existing bands that appear at that location on the gel in the absence of expression of this protein.

Example 3. Human Clinical Study of Truncated Human Type 21 Collagen

[0210] A clinical study using human subjects to determine the effects of a topical skincare product containing truncated human type 21 collagen (SEQ ID NO: 16) is performed. The research is performed according to U.S. and International standards of Good Clinical Practice (FDA and ICH guidelines) and applicable government regulations.

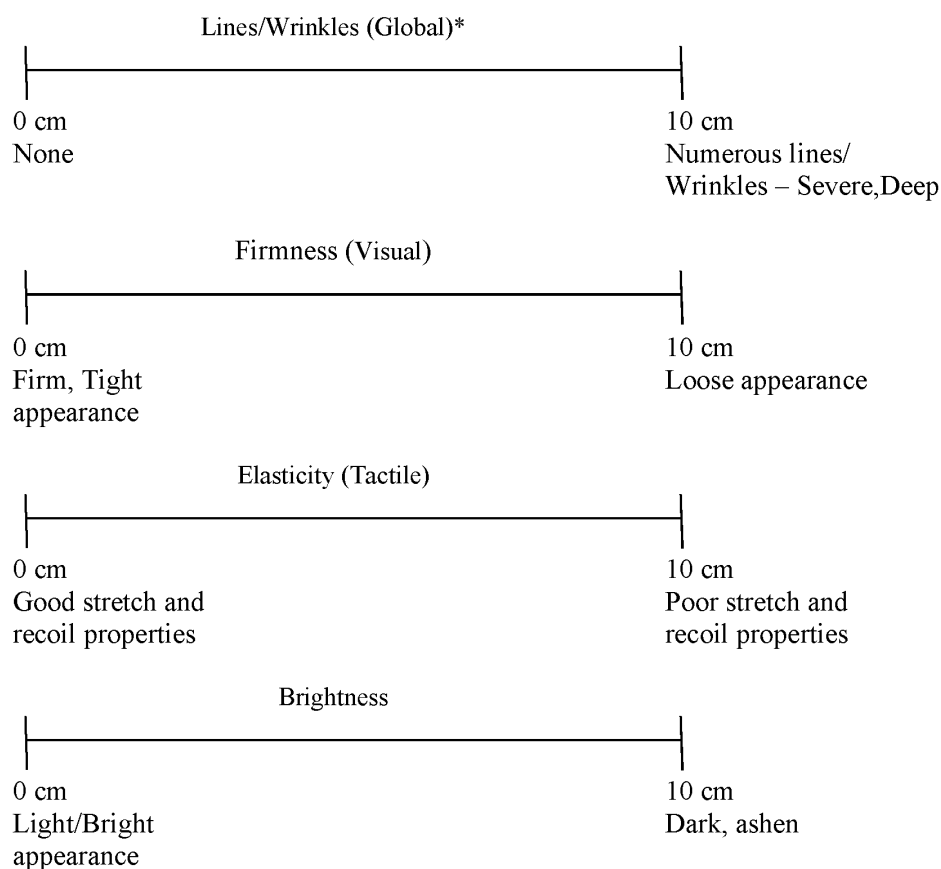
[0211] A base formulation (control formulation) made of water, olive oil glycereth-8 esters, glycerin, coconut alkanes, hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer,

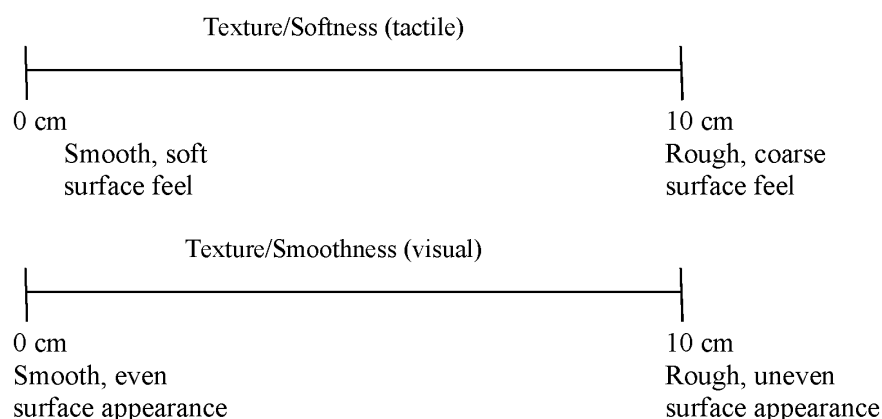
polyethylene glycol, disodium EDTA, caprylyl glycol, chlorphenesin, phenoxyethanol is prepared. A formulation containing the truncated human type 21 collagen is prepared by adding sufficient collagen to prepare a topical formulation containing 0.1% w/w collagen.

Expert Grading Assessments for Qualification and Efficacy

[0212] Visual Analog Scales (VAS) are commonly used in clinical research to measure intensity or frequency of various symptoms, subjective characteristics or attitudes that cannot be directly measured. VAS are a reliable scale and more sensitive to small changes than simple ordinal scales. (A. Paul-Dauphin, F. Guillemin, J. Virion and S. Briancon, "Bias and precision in visual analog scales: A randomized controlled trial," American Journal of Epidemiology, vol. 150, no. 10, pp. 1117-27, 1999). When responding to a VAS item, the expert grader specifies their level of agreement to a statement by indicating a position along a line (10 cm) between two end-points or anchor responses. Simple VAS is used to evaluate efficacy parameters in which the ends of a 10 cm horizontal line is defined as extreme limits orientated from the left (best) to the right (worst). Signs of photo-aging can be classified as follows: Mild=1-3.9 cm, Moderate=4-6.9 cm, Severe=7-10 cm.

[0213] The following VAS was used:





* Inclusion Criteria

[0214] Ordinal scales allow a number to be directly and objectively attached to the quality of a given attribute. When responding to an ordinal scale item, the expert grader specifies their level of agreement to a statement by choosing a set grade, or level.

[0215] The appearance of each subject's facial skin redness (erythema) is assessed by an expert grader using the following five-point ordinal scale for qualification at Baseline (Table 4). If qualified, each subject will undergo further erythema assessments at week 2, week 4, week 6 and week 8.

Table 4. Five-point ordinal scale for erythema assessment

Erythema	
Grade	Description
0	No erythema
1	Very slight erythema (barely perceptible)
2	Well-defined erythema
3	Moderate to severe erythema
4	Severe erythema (beet redness) to slight eschar formation (injuries in depth)

[0216] The corneometer CM 820 (Courage + Khazaka, Germany) measures the relative degree of hydration of the skin surface by applying an alternating current to the skin with a closely spaced pair of electrodes and measuring the capacitance. Changes in water content of the skin change the conductance of the capacitive circuit.

[0217] The corneometer is able to detect slight changes in the hydration level reproducibly with a measurement time of only about one second. The measurement depth is small (approximately 10-20 μm of stratum corneum) which ensures assessment is not influenced by deeper skin layers.

[0218] All subjects undergo corneometer measurements of their face at baseline, immediately post-initial-application, and at weeks 2, 4, and 8. Measurements will be taken in triplicate and

averaged for each time point. Measurement location is recorded on a face map for assessment consistency at each time point.

[0219] The Cutometer MPA 580 (Courage + Khazaka, Germany) measures the viscoelastic properties of the skin by applying suction to the skin surface, drawing the skin into the aperture of the probe and determining the penetration depth using an optical measuring system.

[0220] The resistance of the skin to be sucked up by the negative pressure (firmness) and its ability to return to its original position (elasticity) are calculated and displayed as curves. The Cutometer outputs include many parameters of different portions of the measurement curve including of R0 (Uf, firmness), R2 (Ua/Uf, gross elasticity), R5 (Ur/Ue, net elasticity), R7 (Ur/Uf, elastic portion) and R9 (R3 [last max amp]-R0 [Uf], fatigue).

[0221] All subjects have Cutometer measurements taken on the left or right cheek (following a prepared randomization code) at baseline, immediately post-initial-application, and at weeks 2, 4, and 8. Skin elasticity is reported using the R5 (Ur/Ue) and R2 (Ua/Uf) parameter. As the skin becomes more elastic, this value will increase. Skin Firmness is reported using the R0 (Uf) parameter. As the skin becomes firmer, this value will decrease. Assessment location is recorded on a face map for each subject for consistency of measurements between visits.

[0222] The COSMETRICS™ SIAScope (Astron Clinica, Toft, UK) is a non-invasive optical skin imaging instrument using Spectrophotometric Intracutaneous Analysis (SIA), or chromophore mapping. The technique is based on a unique combination of dermatoscopy and contact remittance spectrophotometry. The hardware consists of a hand-held imaging probe attached to a laptop computer. The unit is placed in contact with the skin surface and high-intensity LED's illuminate the skin as discreet wavelengths of 400 to 1000 nm, spanning the visible spectrum and a small range of the near infrared spectrum. A digital image is captured for each wavelength. Three parametric chromophore maps are retrieved up to 2 mm in depth and 11 mm in circumference, one for each of the following parameters: epidermal melanin, dermal hemoglobin and dermal collagen.

[0223] For the purposes of this study, dermal collagen will be measured on the left or right cheek, per a prepared randomization code, at baseline and at weeks 2, 4, and 8. Assessment location will be recorded on a face map for each subject for consistency of measurements between visits.

[0224] The DermaScan C USB (Cortex Technology ApS, Hadsund, Denmark) is a compact high resolution ultrasound scanner. The 20 MHz, high definition 60 x 150 µm, 13 mm penetration probe is used which provides linear scanning, high precision operation and true position detection for image clarity and definition

[0225] The instrument is provided by cyberDERM, Inc. (Broomall, PA, USA). All subjects have ultrasound assessments taken on the face at baseline and at weeks 2, 4, and 8. The location of assessments is the same at each visit and will be recorded on a face map. Upon acquisition of the ultrasound scans, they are sent to cyberDERM, Inc., for analysis of dermal thickness (density).

[0226] All clinical photography is performed in accordance with IRSI's SOP to ensure reproducibility of high quality images throughout the duration of the study. Imaging is conducted in a designated photography suite with a matte black wall and all natural light is blocked out. To prepare subjects for clinical photography, subjects are asked to remove all jewelry, including earrings, necklaces, and any facial jewelry. A trained technician inspects the subjects under a lighted magnification loop to ensure no residual color cosmetics or skincare products are visible on the face, eyes, or lips. Subjects are provided with a black cape and black headband and are instructed on placement to ensure all hair is pulled back neatly and covered.

[0227] The Clarity™ 2D Research System Ti (Clarity) (BrighTex Bio-Photonics (BTBP), San Jose CA, USA) captures high quality full face frontal, left, and right lateral images. Three cameras within the system allow for 18 megapixel SLR image capture in 16-bit simultaneously using a live feed display and automated facial alignment checks against baseline images for reproducibility.

[0228] Multi-spectral lighting (diffuse white light, cross-polarized, blue and parallel polarized) reveals skin conditions on and beneath the skin's surface layer. The system uses skin feature recognition to apply automated skin segmentation and zone mapping to allow for subsequent skin analysis. Images are analyzed for attributes associated with pigmentation, subsurface pigmentation, radiance, skin color, redness, wrinkles, skin texture, pores, acne, and/or lips.

[0229] All subjects have front, left, and right view facial images captured in standard light and parallel polarized light at baseline and at weeks 2, 4, and 8.

[0230] Subjective questionnaires allow the Sponsor to gauge the subjects' opinions of their skin, the test product, and its effects. Questions will ask for subjects' agreement to a statement with a five- point scale as well as open-ended response.

[0231] Fourteen female subjects are enrolled. The inclusion criteria are Caucasian female subjects with Fitzpatrick skin type III in good general health, and between ages of 35 and 65 years old, inclusive at enrollment. Inclusion criteria also include signs of aging on face as determined by an expert grader at a baseline of: a) Score of ≥ 2 cm ≤ 6 on 10 cm scale for lines/wrinkles; and b) Score of $\geq 1 \leq 3$ on 5-point ordinal scale for facial redness (erythema). Table 5 discloses the demographics of the study participants.

Table 5. Demographics

Variable	n	Mean ± SD	Min	Max
Age (years)	14	57.64 ± 6.03	45	65
Height (inches)	14	63.35 ± 2.37	60	68
Weight (pounds)	14	160.14 ± 38.26	110	250
			n	Percent
Ethnicity	14	Not Hispanic or Latino	14	100%
			n	Percent
Race	14	White	14	100%
			n	Percent
Fitzpatrick Skin Type	14	Skin Type III	14	100%
			n	Percent
Facial Skin Type	14	Combination	8	57.1%
		Normal	6	42.9%

[0232] The results of the expert clinical grader evaluation on lines/wrinkles, firmness (visual), elasticity (tactile), brightness, texture/softness (tactile), texture/smoothness (visual) and erythema after two weeks treatment are shown in Table 6. All of the tested characteristics were improved. The scores for brightness, texture/softness (tactile), texture/smoothness (visual) and erythema improved with statistical significance.

Table 6. Expert Clinical Grader Evaluation – Monadic, Comparison to Baseline

Assessment	Time Point	n	Mean ± SD	Mean Percent Improvement From BL mean	Percent of Subjects Showing Improvement From BL	P-Value TX vs. BL
Lines/ Wrinkles (Global)	Baseline	14	5.22 ± 0.75			
	Week 2	14	5.17 ± 0.75	0.92%	57.1%	0.336
Firmness (Visual)	Baseline	14	5.07 ± 0.77			
	Week 2	14	5.01 ± 0.75	1.08%	64.3%	0.120
Elasticity (Tactile)	Baseline	14	4.91 ± 0.53			
	Week 2	14	4.90 ± 0.52	0.25%	35.7%	0.547
Brightness	Baseline	14	5.51 ± 0.54			
	Week 2	14	5.37 ± 0.62	2.57%	78.6%	0.010*
Texture/ Softness (Tactile)	Baseline	14	4.82 ± 0.82			
	Week 2	14	4.51 ± 0.89	6.43%	71.4%	0.008*
Texture/ Smoothness (Visual)	Baseline	14	5.46 ± 0.70			
	Week 2	14	5.25 ± 0.68	3.63%	64.3%	0.009*
Erythema	Baseline	14	1.60 ± 0.73			
	Week 2	14	1.21 ± 0.80	23.81%	35.7%	0.021*

*Indicates a statistically significant improvement compared to baseline, $p \leq 0.05$

[0233] Instrumental evaluation hydration, firmness elasticity using a corneometer and cutometer are shown in Table 7. Improvements in skin hydration, firmness, and elasticity were statistically significant. In addition, Table 7 shows the stimulation of collagen production by skin cells as demonstrated by Spectrophotometric Intracutaneous Analysis (SIA).

Table 7. Instrumental Evaluation – Monadic, Comparison to Baseline

Assessment		Time Point	n	Mean $\pm$ SD	Mean Percent Improvement From BL mean	Percent of Subjects Showing Improvement From BL	P-Value TX vs. BL
Corneometer		Baseline	14	42.45 $\pm$ 10.06			
		Immediate	14	57.22 $\pm$ 12.47	36.69%	100%	<0.001*
		Week 2	14	35.71 $\pm$ 10.77	NI	14.3%	0.001**
Cutometer	Firmness (R0 Uf)	Baseline	14	0.35 $\pm$ 0.06			
		Immediate	14	0.24 $\pm$ 0.05	30.38%	100%	<0.001*
		Week 2	14	0.31 $\pm$ 0.07	11.27%	78.6%	0.028*
	Elasticity (R2 Ua/Uf)	Baseline	14	0.64 $\pm$ 0.09			
		Immediate	14	0.93 $\pm$ 0.13	45.85%	100%	<0.001*
		Week 2	14	0.80 $\pm$ 0.09	26.93%	100%	<0.001*
	Elasticity (R5 Ur/Ue)	Baseline	14	0.28 $\pm$ 0.06			
		Immediate	14	0.40 $\pm$ 0.09	44.57%	92.9%	<0.001*
		Week 2	14	0.37 $\pm$ 0.08	33.36%	92.9%	<0.001*
SIAscope	Collagen	Baseline	14	238.95 $\pm$ 11.60			
		Week 2	14	254.89 $\pm$ 19.35	6.72%	78.6%	0.004*

NI=No Improvement

*Indicates a statistically significant improvement compared to baseline, $p \leq 0.05$ **Indicates a statistically significant worsening compared to baseline, $p \leq 0.05$

[0234] The results demonstrate that truncated human type 21 collagen shows statistically significant improvements in elasticity, brightness, hydration, tactile texture, or visual texture of skin. In addition, the results show that truncated human type 21 collagen shows statistically significant decreases in visible lines or wrinkles as well as significant decreases in erythema.

Example 4. *In vitro* study of Truncated Human Type 21 Collagen on skin cells

Truncated human type 21 alpha 1 collagen stimulates fibroblast production of Collagen Type I

[0235] A series of *in vitro* experiments were conducted to assess the effects of a truncated human type 21 collagen on human skin fibroblasts and keratinocytes. In a first experiment, human primary fibroblasts were evaluated for collagen type I protein secretion. Fibroblasts were cultured with 0.03% of a polypeptide according to SEQ ID NO: 16 for 48 hours. Culture supernatants were analyzed by Enzyme Linked Immunosorbent Assay (ELISA) for pro-collagen type I C-peptide, which is a readout for total secreted collagen type I protein. As shown in FIG. 1, cells treated with a polypeptide of SEQ ID NO: 16 secreted higher levels of collagen type I (FIG. 1; “B”) than untreated cells (FIG. 1; “A”) or cells treated with retinol (FIG. 1; “C”).

Truncated human type 21 alpha 1 collagen stimulates fibroblast production of genes for extracellular matrix proteins

[0236] RNA sequencing was performed to analyze global gene expression. After 48 hours of exposure, fibroblasts were incubated with 0.03% of a polypeptide according to SEQ ID NO: 16. These fibroblasts expressed higher levels of several extracellular matrix genes than cells incubated in media alone. As shown in FIG. 2A, fibroblasts treated with a polypeptide of SEQ

ID NO: 16 (FIG. 2A; “C”) upregulated the collagen type I gene (COL1A) relative to untreated cells (FIG. 2A; “A”) or fibroblasts treated with retinol (FIG. 2A; “B”). This response was similar to fibroblasts treated with Vitamin C (FIG. 2A; “D”). As shown in FIG. 2B, fibroblasts treated with a polypeptide of SEQ ID NO: 16 (FIG. 2B; “B”) upregulated the elastin gene (ELN) relative to untreated cells (FIG. 2B; “A”), and various marine collagens (FIG. 2B; “C”, “D”, “E”, and “F”). As shown in FIG. 2C, fibroblasts treated with a polypeptide of SEQ ID NO: 16 (FIG. 2C; “B”) upregulated the fibronectin gene (FN1) relative to untreated cells (FIG. 2C; “A”), retinol (FIG. 2C; “C”), and Vitamin C (FIG. 2C; “D”).

Truncated human type 21 alpha 1 collagen reduces inflammation of keratinocytes irradiated with UVB light.

[0237] Human primary keratinocytes were irradiated with 40 mJ/cm² UVB light, and then treated with 0.1% of a polypeptide of SEQ ID NO: 16 for 24 hours. Levels of the pro-inflammatory cytokines IL-1 α were determined by ELISA. As shown in FIG. 3, UVB-irradiated keratinocytes treated with a polypeptide of SEQ ID NO: 16 (FIG. 3; “B”) expressed lower levels of IL-1 α compared to untreated UVB-irradiated keratinocytes (FIG. 3; “A”).

Truncated human type 21 alpha 1 collagen has anti-oxidative capacity.

[0238] The antioxidant potential of a polypeptide of SEQ ID NO: 16 was assessed using the oxygen free radical absorbance capacity (ORAC) assay. The ORAC assay is a cell-free assay that uses a fluorescent readout to measure a product’s antioxidant capacity. Data is reported in Trolox (Vitamin E) equivalents. As shown in FIG. 4, a 0.1% solution of a polypeptide of SEQ ID NO: 16 had antioxidant properties equivalent to 190 μ M Trolox.

Truncated human type 21 alpha 1 collagen increases cell viability of keratinocytes irradiated with UVB light.

[0239] To further assess the effects of treatment with a polypeptide of SEQ ID NO: 16 on UVB-irradiated keratinocytes, an experiment was performed with pre- and post-irradiation treatment. Human primary keratinocytes were pre-treated with 0.1% of a polypeptide of SEQ ID NO: 16 for 24 hours, irradiated with 40 mJ/cm² UVB light, and then treated again with 0.1% of a polypeptide of SEQ ID NO: 16 for an additional 24 hours. Cell viability was evaluated using the MTT metabolic colorimetric assay. As shown in FIG. 5, UVB-irradiated keratinocytes treated with a polypeptide of SEQ ID NO: 16 (FIG. 5; “B”) showed higher cell viability than UVB-irradiated keratinocytes without such treatment (FIG. 5; “A”).

Example 5. Human Clinical Study of Truncated Human Type 21 Collagen.

Topical application of truncated human type 21 alpha 1 collagen is associated with facial skin elasticity increase

[0240] In a clinical study (n=15 subjects), subjects used a topical facial serum containing 0.1% of a polypeptide of SEQ ID NO: 16 for 8 weeks, after using a protein-free base facial serum for a 1-week washout period. Topical application of a polypeptide of SEQ ID NO: 16 was associated with increased skin elasticity, measured using a cutometer. As shown in FIG. 6, 100% of subjects showed improvement with an increase in skin elasticity at 2 weeks (FIG. 6; “B”) and 4 weeks (FIG. 6; “C”) as compared to baseline (FIG. 6; “A”).

Topical application of human type 21 alpha 1 collagen is associated with facial skin collagen content increase

[0241] In a clinical study (n=15 subjects), subjects used a topical facial serum containing 0.1% of a polypeptide of SEQ ID NO: 16 for 8 weeks, after using a protein-free base facial serum for a 1-week washout period. Topical application of a polypeptide of SEQ ID NO: 16 was associated with increased skin collagen content, as measured by a SIAscope. As shown in FIG. 7, skin collagen content increased at 2 weeks (FIG. 7; “B”) and at 8 weeks (FIG. 7; “C”) as compared to baseline (FIG. 7; “A”).

Topical application of human type 21 alpha collagen is associated with a reduction in facial skin redness

[0242] In a clinical study (n=15 subjects), subjects used a topical facial serum containing 0.1% of a polypeptide of SEQ ID NO: 16 for 8 weeks, after using a protein-free base facial serum for a 1-week washout period. As shown in FIG. 8, topical application of a polypeptide of SEQ ID NO: 16 was associated with a decrease in skin redness at 4 weeks (FIG. 8; “B”) and at 8 weeks (FIG. 8; “C”) as compared to baseline (FIG. 8; “A”).

Topical application of human type 21 alpha 1 collagen is associated with a reduction in facial wrinkles

[0243] In a clinical study (n=15 subjects), subjects used a topical facial serum containing 0.1% of a polypeptide of SEQ ID NO: 16 for 8 weeks, after using a protein-free base facial serum for a 1-week washout period. As shown in FIG. 9, topical application of a polypeptide of SEQ ID NO: 16 was associated with a reduction in facial wrinkles at 4 weeks (FIG. 9; “B”) and at 8 weeks (FIG. 9; “C”) as compared to baseline (FIG. 9; “A”).

Example 6. In vitro study of Truncated Jellyfish Collagen on skin cells

Truncated jellyfish collagen stimulates skin cell production of Collagen Type I protein

[0244] A series of in vitro experiments were conducted to assess the effects of a truncated jellyfish collagen on human skin fibroblasts and keratinocytes. An in vitro full thickness human skin tissue model (MatTek) which contains fibroblasts and keratinocytes was evaluated for collagen type I secretion after treatment with a polypeptide of SEQ ID NO: 5 for 48 hours. The

tissue models were then rinsed and incubated with fresh media for another 48 hours (96 hour timepoint). The culture supernatants were analyzed by ELISA for pro-collagen type I C-peptide (a readout for total secreted collagen type I protein). As shown in FIG. 10, the tissue models treated with a polypeptide of SEQ ID NO: 5 (FIG. 10; "A") secreted higher levels of collagen type I than untreated tissue models (FIG. 10; "B") or tissue models treated with the positive control, Vitamin B3 (FIG. 10; "C").

Truncated jellyfish collagen reduces DNA damage in keratinocytes after exposure to UVB light

[0245] In a further study, human primary keratinocytes were irradiated with 25 mJ/cm² UVB light, then incubated overnight in media with 0.03% of a polypeptide of SEQ ID NO: 5. DNA was extracted from the cells and analyzed for levels of thymine dimers (an indicator of DNA damage) using an OxiSelect UV-Induced DNA Damage ELISA kit. As shown in FIG. 11, cells treated with a polypeptide of SEQ ID NO: 5 (FIG. 11; "B") showed a lower level of thymine dimers, and thus less DNA damage, than untreated cells (FIG. 11; "A").

Truncated jellyfish collagen increases cell viability of keratinocytes irradiated with UVB light

[0246] Human primary keratinocytes were irradiated with 40 mJ/cm² UVB light, then incubated for 48 hours in media with 0.03% of a polypeptide of SEQ ID NO: 5. Cell viability was evaluated using the MTT metabolic colorimetric assay. As shown in FIG. 12, UVB-irradiated keratinocytes treated with a polypeptide of SEQ ID NO: 5 (FIG. 12; "B") showed higher cell viability than untreated UVB-irradiated keratinocytes (FIG. 12; "A").

Truncated jellyfish collagen increases cell viability of keratinocytes exposed to urban dust pollution

[0247] To test for protection from urban dust, human primary keratinocytes were pre-treated with 0.03% of a polypeptide of SEQ ID NO: 5 for 24 hours, and then exposed to 2 mg/ml urban dust (NIST 1649B) for 24 hours. Cell viability was evaluated using the MTT metabolic colorimetric assay. As shown in FIG. 13, keratinocytes pre-treated with a polypeptide of SEQ ID NO: 5 (FIG. 13; "B") showed higher cell viability after urban dust exposure than untreated keratinocytes exposed to urban dust (FIG. 13; "A").

Truncated jellyfish collagen reduces inflammation of keratinocytes irradiated with UVB light

[0248] In a further study with the in vitro full thickness human skin tissue model (MatTek), the MatTek tissue models were irradiated with 300 mJ/cm² UVB light, then treated with 0.01% of a polypeptide of SEQ ID NO: 5 for 24 hours. Levels of pro-inflammatory cytokine IL-1 α was

determined by ELISA. As seen in FIG. 14, the tissue model treated with a polypeptide of SEQ ID NO: 5 (FIG. 14; “A”) showed lower levels of IL-1 α compared to the untreated UVB-irradiated (FIG. 14; “B”) control tissue model.

Truncated jellyfish collagen has anti-oxidative capacity

[0249] A polypeptide of SEQ ID NO: 5 was also evaluated in the ORAC assay. As shown in FIG. 15, a 0.1% solution of a polypeptide of SEQ ID NO: 5 had anti-oxidative properties equivalent to 193 μ M Trolox.

Example 7. Human Clinical Study of Truncated Jellyfish Collagen

Topical application of a truncated jellyfish collagen is associated with an increase in facial skin moisture

[0250] In a clinical study (n=18 subjects), subjects used a topical facial cream containing 0.05% of a polypeptide of SEQ ID NO: 5 for 2 weeks. As shown in FIG. 16, topical application of a polypeptide of SEQ ID NO: 5 was associated with increased skin hydration at 1 week (FIG. 16; “A2”) and at 2 weeks (FIG. 16; “A3”) as compared to baseline (FIG. 16; “A1”). Topical application of a polypeptide of SEQ ID NO: 5 also demonstrated increased skin hydration relative to topical application of marine collagen at baseline (FIG. 16; “B1”), at 1 week (FIG. 16; “B2”), and at 2 weeks (FIG. 16; “B3”).

Topical application of truncated jellyfish collagen is associated with an increase in facial skin elasticity

In a clinical study (n=18 subjects), subjects used a topical facial cream containing 0.05% of a polypeptide of SEQ ID NO: 5 for 2 weeks. As shown in FIG. 17, topical application of a polypeptide of SEQ ID NO: 5 was associated with increased skin elasticity, measured using a cutometer, at 1 week (FIG. 17; “B”) and at 2 weeks (FIG. 17; “C”), as compared to baseline (FIG. 17; “A”).

[0251] The disclosed embodiment herein may be embodied in other specific forms without departing from the structures, methods, or other characteristics as broadly described herein and claimed hereinafter. The described embodiments are to be considered in all respects only as illustrative, and not restrictive. All changes that come within the meaning and range of equivalency of the claims are to be embraced within their scope.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of increasing the firmness, elasticity, brightness, hydration, tactile texture, collagen content, elastin content, reducing redness, or visual texture of skin, the method comprising topically applying a non-naturally occurring truncated collagen to the skin (e.g., wherein the truncated collagen is a polypeptide comprising an amino acid sequence that is or comprises an amino acid sequence that is truncated relative to the amino acid sequence of a naturally occurring collagen, such as a naturally occurring human or jellyfish collagen).
2. A method of decreasing lines or wrinkles present on skin or decreasing erythema of skin, the method comprising topically applying a non-naturally occurring truncated collagen to the skin (e.g., wherein the truncated collagen is a polypeptide comprising an amino acid sequence that is or comprises an amino acid sequence that is truncated relative to the amino acid sequence of a naturally occurring collagen, such as a naturally occurring human or jellyfish collagen).
3. The method of claim 1 or 2, wherein the non-naturally occurring truncated collagen is a human collagen or a jellyfish collagen.
4. The method of claim 1 or 3, wherein the truncated collagen is a human collagen.
5. The method of claim 3, wherein the human collagen is truncated at the C-terminal end, the N-terminal end, internally truncated, or truncated at both the C-terminal end and the N-terminal end.
6. The method of claim 5, wherein the human collagen is truncated at both the C-terminal end and the N-terminal end.
7. The method of claim 6, wherein the collagen is truncated at the C-terminal end by between 10 and 800 amino acids and/or truncated at the N-terminal end by between 10 and 800 amino acids.
8. The method of any of claims 3 to claim 7, wherein the truncated collagen is between 10 and 900 amino acids in length, between 10 and 800 amino acids in length, between 10 and 700 amino acids in length, between 10 and 600 amino acids in length, between 10 and 500 amino acids in length, between 10 and 400 amino acids in length, between 10 and 300 amino acids in length, between 10 and 200 amino acids in length, between 10 and 100 amino acids in length, between 10 and 50 amino acids in length, between 50 and 800 amino acids in length, between 50 and 700 amino acids in length, between 50 and 600 amino acids in length, between 50 and 500 amino acids in length, between 50 and 400 amino acids in length, between

50 and 300 amino acids in length, between 50 and 200 amino acids in length, or between 50 and 100 amino acids in length.

9. The method of any of claims 1 to claim 8, wherein the collagen is a truncated human type 21 collagen.

10. The method of claim 9, wherein the truncated human type 21 collagen is SEQ ID NO: 16

11. The method of any of claim 1 to claim 10, wherein the firmness of the skin increases by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70% or 75% when measured using a cutometer.

12. The method of any of claim 1 to claim 10, wherein the elasticity of the skin increases by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70% or 75% when measured on a cutometer.

13. The method of claim 12, wherein the elasticity of the skin increases by at least 8% when measured on a cutometer after 1 week of treatment.

14. The method of claim 12, wherein the elasticity of the skin increases by at least 25% when measured on a cutometer after 2 weeks of treatment.

15. The method of claim 12, wherein the elasticity of the skin increases by at least 30% when measured on a cutometer after 2 weeks of treatment.

16. The method of claim 12, wherein the elasticity of the skin increases by at least 100% when measured on a cutometer after 4 weeks of treatment.

17. The method of either one of claims 13 or 16, wherein the non-naturally occurring truncated collagen to the skin is applied as facial serum comprising 0.1% w/w of the non-naturally occurring truncated collagen.

18. The method of any of claim 1 to claim 10, wherein the hydration of the skin increases by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70% or 75% when measured on a corneometer.

19. The method of any of claim 1 to claim 10, wherein the firmness of the skin is improved as determined by an expert clinical grader.

20. The method of any of claim 1 to claim 10, wherein the elasticity of the skin is improved as determined by an expert clinical grader.

21. The method of any of claim 1 to claim 10, wherein the brightness of the skin is improved as determined by an expert clinical grader.

22. The method of any of claim 1 to claim 10, wherein the collagen content of the skin is increased by at least 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% when measured on a SIAscope.

23. The method of claim 22, wherein the collagen content of the skin is increased by at least 5% after two weeks.

24. The method of claim 22, wherein the collagen content of the skin is increased by at least 7% after two weeks.

25. The method of claim 22, wherein the collagen content of the skin is increased by at least 9% after four weeks.

26. The method of any of claim 1 to claim 10, wherein the tactile texture of the skin is improved as determined by an expert clinical grader.

27. The method of any of claim 1 to claim 10, wherein the visual texture of the skin is improved as determined by an expert clinical grader.

28. The method of any of claim 1 to claim 10, wherein the lines or wrinkles present on skin is decreased as determined by an expert clinical grader.

29. The method of claim 28, wherein the lines or wrinkles present on skin is decreased by at least 10% as determined by an expert clinical grader. The method of any of claim 1 to claim 10, wherein the erythema of the skin is decreased as determined by an expert clinical grader.

30. The method of claim 29, wherein the erythema of the skin is decreased by at least 10%, 20%, 30%, 40%, or 45%, as determined by an expert clinical grader, after 2 weeks of topical application.

31. The method of claim 29, wherein the erythema of the skin is decreased by at least 10%, 20%, 30%, 40%, 45%, 50%, or 55%, as determined by an expert clinical grader, after 4 weeks of topical application.

32. A method of stimulating collagen production in a skin cell, the method comprising topically applying a non-naturally occurring truncated collagen to the skin.

33. The method of claim 32, wherein the non-naturally occurring truncated collagen is a human collagen.

34. The method of claim 33, wherein the human collagen is truncated at the C-terminal end, the N-terminal end, internally truncated, or truncated at both the C-terminal end and the N-terminal end.

35. The method of claim 34, wherein the human collagen is truncated at both the C-terminal end and the N-terminal end.

36. The method of claim 35, wherein the collagen is truncated at the C-terminal end by between 10 and 800 amino acids and/or truncated at the N-terminal end by between 10 and 800 amino acids.

37. The method of any of claims 33 to claim 36, wherein the truncated collagen is between 10 and 900 amino acids in length, between 10 and 800 amino acids in length, between 10 and 700 amino acids in length, between 10 and 600 amino acids in length, between 10 and 500 amino acids in length, between 10 and 400 amino acids in length, between 10 and 300 amino acids in length, between 10 and 200 amino acids in length, between 10 and 100 amino acids in length, between 10 and 50 amino acids in length, between 50 and 800 amino acids in length, between 50 and 700 amino acids in length, between 50 and 600 amino acids in length, between 50 and 500 amino acids in length, between 50 and 400 amino acids in length, between 50 and 300 amino acids in length, between 50 and 200 amino acids in length, or between 50 and 100 amino acids in length.

38. The method of any of claims 32 to claim 37, wherein the collagen is a truncated human type 21 collagen.

39. The method of claim 38, wherein the truncated human type 21 collagen is SEQ ID NO: 16.

40. The method of any of claims 32 to claim 39, wherein the collagen in the skin increases by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, or at least 10%.

41. The method of any of claims 32 to claim 39, wherein the collagen in the skin increases by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, or at least 10%.

42. A topical formulation comprising a truncated human type 21 collagen and one or more additional ingredient selected from the group consisting of water, oil glycereth-8 esters, glycerin, coconut alkanes, hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, pentyleneglycol, disodium EDTA, caprylyl glycol, chlorphenesin, and phenoxyethanol.

43. The topical formulation of claim 42, wherein the truncated human type 21 collagen is SEQ ID NO: 16.

44. The topical formulation of claim 42 or claim 43, wherein the oil is a vegetable oil, preferably olive oil.

45. The topical formulation of claim 43 or claim 44, wherein said truncated human type 21 collagen upregulates elastin expression when applied to a human primary fibroblasts to a greater level than a marine collagen does when applied to human primary fibroblasts.

46. A topical formulation comprising a truncated jellyfish collagen and one or more additional ingredient selected from the group consisting of water, oil glycereth-8 esters, glycerin, coconut alkanes, hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, pentyleneglycol, disodium EDTA, caprylyl glycol, chlorphenesin, and phenoxyethanol.

47. The topical formulation of claim 46, wherein the truncated jellyfish is SEQ ID NO: 5.

48. The topical formulation of claim 46 or claim 47, wherein the oil is a vegetable oil, preferably olive oil.