

RECOMBINANT COLLAGEN AND PREPARATION METHOD AND USE THEREOF

TECHNICAL FIELD

The present disclosure relates to a recombinant collagen and a preparation method and use thereof, and in particular to a full-length collagen $\alpha 1$ chain produced through recombinant expression, and a preparation method and use thereof. The present disclosure belongs to the technical field of collagen expression.

BACKGROUND

Type I collagen and type II collagen are typical fibrillar collagens in the human body. Type I collagen and type II collagen each are composed of three α peptide chains, and each α peptide chain contains an amino-terminal peptide region, a characteristic (G-X-Y)_n triplet-repeat sequence region, and a carboxyl-terminal peptide region.

Type I collagen is composed of two $\alpha 1$ chains and one $\alpha 2$ chain. Type I collagen is the most abundant collagen among all types of collagen in the human body, and is found in muscles, skins, arterial walls, and fibrocartilage. Type II collagen is composed of three $\alpha 1$ chains, and is mainly distributed in cartilage tissues, vitreous humors, and corneas. The amount of type II collagen accounts for 90% or more of the total amount of collagen in an adult cartilage matrix. Type II collagen is an essential component for chondrogenic and skeletal pattern formation, skeletal growth, and mature cartilage maintenance.

As an important native biological protein, collagen has unique functional characteristics such as excellent biocompatibility, biological activity, and degradability. Therefore, collagen can be widely used in many fields such as chemical industry, medicine, food, and cosmetics, and is especially suitable for the preparation of various biological devices. Collagen is the most desirable source of biological materials and has promising application prospects.

Commercially available collagen products are mainly collagen extracts obtained by treating animal tissues with acid hydrolysis, alkaline hydrolysis, and enzymatic hydrolysis. However, during the above treatment process, collagen is severely degraded and thus loses its biological activity, and extracted collagen peptides have different lengths, heterogeneous properties, unstable qualities, and potential safety hazards from viral infections such as bovine spongiform encephalopathy and foot-and-mouth disease. In addition, animal-derived collagen has a quite different amino acid sequence from human-derived collagen, and is a heterologous protein, which will lead to immune rejection and allergic symptoms.

The production of a recombinant collagen through genetic engineering can effectively avoid these defects. Among the existing expression methods for recombinant collagen, expression systems such as mammalian cell expression systems, insect cell (baculovirus) expression

systems, and transgenic animal and plant expression systems have characteristics such as high costs, low yield, and long cycle, and are mostly used for experiments at a scientific research stage. In large-scale industrial production, prokaryotic (*Escherichia coli*) expression systems and *Pichia pastoris* expression systems are mainly used to express human collagen. In *Escherichia coli*, there is no post-translational modification in the protein, and the protein is expressed intracellularly on a large scale. As a result, the bacterial cells need to be lysed, such that a large number of impurities such as host proteins and native endotoxins and peptidoglycans (as cell wall components) are produced, which can only be removed by a complicated purification process. Because human collagen is a foreign protein for *Pichia pastoris* after all, the expression of human collagen in *Pichia pastoris* occupies many intracellular resources (the methanol metabolism pathway relied by the above expression can result in the expression of up to 30% of soluble proteins in the cell), and *Pichia pastoris* cells will adjust correspondingly in response to a foreign protein. Typically, the recombinant protein is severely degraded in *Pichia pastoris*. $\alpha 1$ chains of human type I collagen and type II collagen each are a long peptide chain of 1,000 or more amino acids, and thus are prone to degradation.

$\alpha 1$ chains of human type I collagen and type II collagen each contain an amino-terminal peptide, a triple helix region, and a carboxyl-terminal peptide in a mature sequence thereof. A human type I collagen $\alpha 1$ chain (hereinafter canonically referred to as $\alpha 1$ (I)) has a full length of 1,057 amino acids (AA), and a human type II collagen $\alpha 1$ chain (hereinafter canonically referred to as $\alpha 1$ (II)) has a full length of 1,060 AA. There are many studies and patents on the expression of the human $\alpha 1$ (I) chain in *Pichia pastoris*, and few studies and patents on the expression of the human $\alpha 1$ (II) chain. In most of the existing studies on the expression of a full-length $\alpha 1$ (I) chain and a full-length $\alpha 1$ (II) chain in *Pichia pastoris*, only a part of the sequence of the $\alpha 1$ (I) chain, rather than the mature full-length sequence of the $\alpha 1$ (I) chain, is expressed. In some published findings, although a full-length $\alpha 1$ (I) chain is expressed, a main degradation product in substantially the same proportion as a target product is produced during the expression, and thus a main degradation band (the main degradation product) in substantially the same proportion as a target band (the target product) of the full-length $\alpha 1$ chain is presented during sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Such degradation results in reduced yield of the full-length $\alpha 1$ chain expressed. In addition, because the main degradation product has similar correlated properties to the full-length $\alpha 1$ chain, two-step tandem affinity purification is required to obtain a high-purity single full-length $\alpha 1$ chain product, which increases the complexity of the purification process and increases purification costs accordingly. Therefore, an important challenge for the production of a recombinant collagen in *Pichia pastoris* is to keep the full-length peptide chain intact and reduce the degradation, while maintaining the biological activity of the collagen unchanged.

SUMMARY

An objective of the present disclosure is to overcome some technical problems in the prior art, and to provide a full-length collagen $\alpha 1$ chain produced through recombinant expression in *Pichia pastoris*, and a preparation method and use thereof. Compared with native full-length $\alpha 1$ (I) and $\alpha 1$ (II) chains, when a variant of a recombinant human type I collagen $\alpha 1$ chain (which is denoted as $\alpha 1$ (I) M1) and a variant of a recombinant human type II collagen $\alpha 1$ chain (which is denoted as $\alpha 1$ (II) M6) according to the present disclosure are expressed in *Pichia pastoris*, a main degradation band (a main degradation product) in substantially the same proportion as a target band (a target product) of a full-length $\alpha 1$ chain is eliminated, and a yield of the target product is improved. The variants according to the present disclosure have similar physical and chemical characteristics and identical biological activity to the native full-length collagen $\alpha 1$ (I) and $\alpha 1$ (II) chains and commercially available human collagens expressed in *Pichia pastoris*, and thus both have application values in the field of biomedical materials.

To achieve the above objective, the present disclosure adopts the following technical solutions.

The present disclosure provides a recombinant collagen $\alpha 1$ chain, where the recombinant collagen $\alpha 1$ chain is $\alpha 1$ (I) M1 or $\alpha 1$ (II) M6; and the $\alpha 1$ (I) M1 is obtained through an amino acid mutation in a native full-length amino acid sequence of a human type I collagen $\alpha 1$ chain, and the $\alpha 1$ (II) M6 is obtained through an amino acid mutation in a native full-length amino acid sequence of a human type II collagen $\alpha 1$ chain.

Preferably, the $\alpha 1$ (I) M1 has 4 amino acid mutation sites, and the $\alpha 1$ (II) M6 has 9 amino acid mutation sites.

Preferably, the human type I collagen $\alpha 1$ chain is set forth in SEQ ID NO: 1; and the amino acid mutation sites include M at position 106, R at position 109, M at position 190, and R at position 193, and specifically, the amino acid mutation sites are mutated to P.

The human type II collagen $\alpha 1$ chain is set forth in SEQ ID NO: 4; and the amino acid mutation sites include V at position 67, M at position 68, M at position 72, M at position 75, R at position 78, M at position 108, R at position 111, M at position 162, and R at position 165, and specifically, the amino acid mutation sites are mutated to P.

Further, the $\alpha 1$ (I) M1 has an amino acid sequence as set forth in SEQ ID NO: 2, and the $\alpha 1$ (II) M6 has an amino acid sequence as set forth in SEQ ID NO: 5.

In the present disclosure, when the amino acid mutations at the amino acid mutation sites of the amino acid sequence are changed to some extent, that is, an amino acid(s) at the same amino acid mutation site(s) of the amino acid sequence is mutated to a different amino acid(s), technical effects similar to those of the present disclosure can also be obtained. When one or more of the

amino acid mutation sites is/are changed, technical effects similar to those of the present disclosure may also be obtained.

The present disclosure also provides a nucleotide encoding the recombinant collagen $\alpha 1$ chain, where a sequence of the nucleotide encoding the recombinant collagen $\alpha 1$ chain includes a nucleotide sequence encoding the $\alpha 1$ (I) M1 or a nucleotide sequence encoding the $\alpha 1$ (II) M6.

Further, the nucleotide sequence encoding the $\alpha 1$ (I) M1 is set forth in SEQ ID NO: 3; and the nucleotide sequence encoding the $\alpha 1$ (II) M6 is set forth in SEQ ID NO: 6.

The present disclosure also provides a recombinant expression vector containing the nucleotide encoding the recombinant collagen $\alpha 1$ chain.

The present disclosure also provides an engineered strain constructed with the recombinant expression vector, where the engineered strain carries the recombinant expression vector or expresses the recombinant collagen $\alpha 1$ chain.

A host strain for the engineered strain is preferably *Pichia pastoris*. The engineered strain was deposited in the China General Microbiological Culture Collection Center (CGMCC) located at NO. 1, West Beichen Road, Chaoyang District, Beijing, China on March 11, 2021, with an accession number of CGMCC NO. 21891 or CGMCC NO. 21892 and a taxonomic name of *Pichia pastoris*. The engineered strain with the accession number of CGMCC NO. 21891 expresses the recombinant collagen $\alpha 1$ chain $\alpha 1$ (I) M1, and the engineered strain with the accession number of CGMCC NO. 21892 expresses the recombinant collagen $\alpha 1$ chain $\alpha 1$ (II) M6.

It should be noted that the host strain of the present disclosure is not limited to *Pichia pastoris*. According to the method of the present disclosure, the recombinant collagen $\alpha 1$ chain can be produced by secretory expression in *Pichia pastoris* or other yeast species, and technical effects similar to those of the present disclosure can theoretically be obtained.

The present disclosure also provides a use of the recombinant expression vector or the engineered strain in an expression of the recombinant collagen $\alpha 1$ chain.

The present disclosure also provides a preparation method of the recombinant collagen $\alpha 1$ chain, including:

(1) Synthesizing a nucleotide sequence encoding the recombinant collagen $\alpha 1$ chain;

where respective amino acids at amino acid mutation sites of an amino acid sequence of a native collagen $\alpha 1$ chain are mutated. 4 amino acids of a type I collagen $\alpha 1$ chain are mutated to obtain $\alpha 1$ (I) M1, and 9 amino acids of a type II collagen $\alpha 1$ chain are mutated to obtain $\alpha 1$ (II) M6. Affinity purification tags are added to an amino terminus and a carboxyl terminus of the amino acid sequence, and then a DNA sequence encoding $\alpha 1$ (I) M1 or $\alpha 1$ (II) M6 is synthesized, such that the DNA sequence includes bispecific affinity purification tags, which facilitates the immunological antibody detection based on the two tag sequences.

Detection results show that, when the $\alpha 1$ (I) M1 or $\alpha 1$ (II) M6 is expressed in *Pichia pastoris*, a main degradation band (a main degradation product) in substantially the same proportion as a target band (a target product) of a full-length $\alpha 1$ chain produced when the full-length $\alpha 1$ (I) chain or full-length $\alpha 1$ (II) chain is expressed in *Pichia pastoris* is eliminated.

Compared with the original native sequences, mutated amino acids in the $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 are located in a characteristic (G-X-Y)_n triplet-repeat sequence region, but all are amino acids located on X and Y, such that structural characteristics of an amino acid sequence of the (G-X-Y)_n triplet-repeat sequence in collagen do not change. In addition, the $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 still maintain physical and chemical characteristics and biological activities similar to those of the original collagen.

(2) Constructing a recombinant expression vector;

where the synthesized DNA sequences are ligated to expression vectors pPIC9K to construct a recombinant expression vector pPIC9K-COL1A1M1 expressing the recombinant collagen $\alpha 1$ (I) M1 and a recombinant expression vector pPIC9K-COL2A1M6 expressing the recombinant collagen $\alpha 1$ (II) M6, respectively.

(3) Constructing recombinant engineered strains for an inducible expression, and screening the recombinant engineered strains;

where the recombinant expression vector is linearized with Sac I and then electrotransformed into *Pichia pastoris* competent cells, the electrotransformed *Pichia pastoris* competent cells are transferred to a Minimal Dextrose (MD) plate for primary screening and then further screened on Yeast Extract Peptone Dextrose (YPD) plates with different concentrations of G418, and then colonies are picked and inoculated into a Buffered Glycerol-complex Medium (BMGY medium) and subjected to inducible expression in a Buffered Methanol-complex Medium (BMMY medium). An engineered strain with a high expression level is screened out.

The engineered strain with the high expression level screened out is *Pichia pastoris*, and has an accession number of CGMCC NO. 21891 or CGMCC NO. 21892.

(4) Cultivating for high-density fermentation;

where the engineered strain with the high expression level identified through protein expression is cultivated in a fermentation tank for a high-density fermentation to obtain a fermentation supernatant.

(5) Purifying the fermentation supernatant to obtain a protein;

where the fermentation supernatant is purified by one-step cation exchange chromatography and then lyophilized to obtain a high-purity collagen $\alpha 1$ (I) M1 or $\alpha 1$ (II) M6.

The obtained recombinant collagen $\alpha 1$ (I) M1 and recombinant collagen $\alpha 1$ (II) M6 expressed in *Pichia pastoris* according to the present disclosure are analyzed through protein property characterization and *in vitro* experiments. The recombinant collagen $\alpha 1$ (I) M1 and

recombinant collagen $\alpha 1$ (II) M6 obtained in the present disclosure have structural characteristics of recombinant collagen, have cell adhesion activities, and are substantially consistent with the commercially available human collagens. More importantly, the two variant proteins of the present disclosure have similar or identical structural characteristics and cell adhesion activities compared with the unmutated original collagen.

The present disclosure also provides a composition including the recombinant collagen $\alpha 1$ chain or a collagen $\alpha 1$ chain prepared by the preparation method described above.

The present disclosure also provides an article including the recombinant collagen $\alpha 1$ chain or a collagen $\alpha 1$ chain prepared by the preparation method described above or the composition described above. The article includes, but is not limited to, a drug, a pharmaceutical composition, a medical device, a biological material, a tissue-engineered product, a cosmetic, or a health product.

Further, the article includes a material for providing an adhesion, a support, and a growth and migration space for a cell or a material serving as a channel for delivering a nutrient and a metabolite.

Further, the article is a collagen hydrogel.

The present disclosure also provides a use of the recombinant collagen $\alpha 1$ chain, the nucleotide, the recombinant expression vector, the engineered strain, or the composition in the manufacture of a product, where the product includes, but is not limited to, a drug, a medical device, a biological material, a tissue-engineered product, a cosmetic, or a health product.

The present disclosure also provides a use of the recombinant collagen $\alpha 1$ chain, the nucleotide, the recombinant expression vector, the engineered strain, or the composition in the manufacture of a product for promoting wound healing or tissue regeneration. Further, the product is a collagen hydrogel.

The present disclosure has the following advantages.

(1) In the collagen $\alpha 1$ chain variants of the present disclosure, a proportion of the amino acid mutation sites in the original native sequence is very small (the proportion of mutated amino acids is lower than 1%, and a homology between amino acid sequences before and after mutation is higher than 99%), such that the complete recombinant $\alpha 1$ chain collagen is obtained without changing the properties (physical and chemical characteristics and biological activity) of the original protein itself, and the collagen $\alpha 1$ chain variants of the present disclosure have the same properties and biological activities as a recombinant protein of a native sequence when prepared into related products.

The $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 have physical and chemical characteristics and biological activities similar to those of $\alpha 1$ (I) and $\alpha 1$ (II), respectively, and both have application values in the field of biomedical materials. In the present disclosure, it is found through cell adhesion

experiments on $\alpha 1$ (I) M1, $\alpha 1$ (II) M6, $\alpha 1$ (I), and $\alpha 1$ (II) that, there is no significant difference in the cell adhesion activity between $\alpha 1$ (I) and $\alpha 1$ (I) M1 and between $\alpha 1$ (II) M6 and $\alpha 1$ (II), and the cell adhesion activities of the $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 are substantially consistent with those of the commercially available human collagens. Collagen hydrogels are prepared from collagens $\alpha 1$ (I) M1, $\alpha 1$ (II) M6, $\alpha 1$ (I), and $\alpha 1$ (II) expressed in *Pichia pastoris*, and hydromechanical characteristics of the collagen hydrogels are tested. There is no significant difference in the viscosity, elasticity modulus, and swelling degree between the collagen hydrogel prepared from $\alpha 1$ (I) and the collagen hydrogel prepared from $\alpha 1$ (I) M1 and between the collagen hydrogel prepared from $\alpha 1$ (II) M6 and the collagen hydrogel prepared from $\alpha 1$ (II). Lyophilized collagen hydrogels are scanned by scanning electron microscopy (SEM). These collagen hydrogels all have a porous network structure and a pore size ranging from 100 μm to 200 μm , and have the potential to be applied in the field of biomedical materials. The four collagen hydrogels each are co-cultivated with NIH/3T3 cells *in vitro*. After Calcein Acetoxymethyl Ester (Calcein AM) is added, viable cells exhibiting green fluorescence that adhere to the collagen hydrogel and grow in the collagen hydrogel can be detected. After methylthiazolyldiphenyl-tetrazolium bromide (MTT) is added, blue-purple crystals produced by viable cells that adhere to the collagen hydrogel and migrate and grow into the collagen hydrogel can be observed.

(2) In the present disclosure, expressed proteins are identified by SDS-PAGE and Western blot (WB). Identification results show that, compared with the full-length $\alpha 1$ (I) chain and full-length $\alpha 1$ (II) chain, when the $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 are expressed in *Pichia pastoris*, a main degradation band (a main degradation product) in substantially the same proportion as a target band (a target product) of a full-length $\alpha 1$ chain is eliminated, and the yield of the target product is improved. In addition, a high-density fermentation experiment is performed in a fermentation tank, and fermentation products are detected by SDS-PAGE. It can be found that $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 each can still maintain the integrity of the target band and do not lead to a main degradation band under high-density fermentation conditions, while the recombinant human $\alpha 1$ (I) and $\alpha 1$ (II) produced by fermentation under the same high-density fermentation conditions lead to obvious main degradation bands.

Moreover, for the recombinant collagen of the present disclosure, the fermentation supernatant only needs to be purified by one-step cation exchange chromatography and then lyophilized to obtain the lyophilized high-purity $\alpha 1$ (I) M1 or $\alpha 1$ (II) M6 collagen sponge. The collagen sponge is detected by SDS-PAGE, and there is mainly a single band of the target product, and no main degradation band, indicating that the high-purity target product is obtained. In the present disclosure, the cost of purification is reduced. However, for $\alpha 1$ (I) and $\alpha 1$ (II), after the fermentation supernatant is purified by one-step cation exchange chromatography, a purified protein product that is a mixture of a target band (a target product, a full-length $\alpha 1$ chain) and a

main degradation band (a main degradation product) can only be obtained. Thus, two-step affinity chromatography is required to obtain the high-purity collagen $\alpha 1$ (I) or $\alpha 1$ (II).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows differences between amino acid sequences of $\alpha 1$ (I) M1 and $\alpha 1$ (I), where amino acids in bold and gray background indicate difference sites.

FIG. 2 shows differences between amino acid sequences of $\alpha 1$ (II) M6 and $\alpha 1$ (II), where amino acids in bold and gray background indicate difference sites.

FIG. 3 is a map of a pPIC9K-COL1A1M1 vector.

FIG. 4 is a map of a pPIC9K-COL2A1M6 vector.

FIG. 5 shows an SDS-PAGE pattern of supernatants produced after inducible expression of $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 collagens for 24 h.

FIG. 6 shows WB patterns of supernatants produced after inducible expression of $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 collagens for 24 h, where the left panel is a WB pattern with an anti-6 $\times$ His Tag antibody and the right panel is a WB pattern with an anti-Strep-Tag II antibody.

FIG. 7 shows mass spectrometry results of target bands and main degradation bands in SDS-PAGE results of $\alpha 1$ (I) and $\alpha 1$ (II) collagens.

FIG. 8 shows mass spectrometry results of target bands in SDS-PAGE results of $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 collagens.

FIG. 9 shows SDS-PAGE patterns of fermentation supernatants produced after inducible expression of $\alpha 1$ (I) M1, $\alpha 1$ (II) M6, $\alpha 1$ (I), and $\alpha 1$ (II) collagens for 48 h.

FIG. 10 shows SDS-PAGE patterns of purified and lyophilized $\alpha 1$ (I) M1, $\alpha 1$ (II) M6, $\alpha 1$ (I), and $\alpha 1$ (II) sponges.

FIG. 11 shows Fourier transform infrared (FT-IR) spectroscopy results of $\alpha 1$ (I) and $\alpha 1$ (II) collagens.

FIG. 12 shows FT-IR spectroscopy results of $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 collagens.

FIG. 13 shows cell adhesion activity results of $\alpha 1$ (I) M1, $\alpha 1$ (II) M6, $\alpha 1$ (I), and $\alpha 1$ (II) collagens.

FIG. 14 shows SEM images of surfaces of lyophilized $\alpha 1$ (I) and $\alpha 1$ (I) M1 collagen hydrogels (the two upper images show the $\alpha 1$ (I) collagen hydrogel and the two lower images show the $\alpha 1$ (I) M1 collagen hydrogel).

FIG. 15 shows SEM images of surfaces of lyophilized $\alpha 1$ (II) and $\alpha 1$ (II) M6 collagen hydrogels (the two upper images show the $\alpha 1$ (II) collagen hydrogel and the two lower images show the $\alpha 1$ (II) M6 collagen hydrogel).

FIG. 16 shows results of adherent growth of NIH/3T3 cells in $\alpha 1$ (I) and $\alpha 1$ (I) M1 collagen hydrogels,

where the three upper images show the following from left to right, respectively: NIH/3T3 cells growing adherently on the $\alpha 1$ (I) collagen hydrogel (the image being taken with a brightfield microscope), NIH/3T3 cells growing adherently on the $\alpha 1$ (I) collagen hydrogel (stained with Calcein AM, bright parts show cells exhibiting green fluorescence, and the image being taken with a fluorescence microscope), and blue-purple crystals produced by NIH/3T3 cells growing in the $\alpha 1$ (I) collagen hydrogel (stained with MTT, dark parts show the crystals, and the image being taken with a brightfield microscope); and

the three lower images show the following from left to right, respectively: NIH/3T3 cells growing adherently on the $\alpha 1$ (I) M1 collagen hydrogel (the image being taken with a brightfield microscope), NIH/3T3 cells growing adherently on the $\alpha 1$ (I) M1 collagen hydrogel (stained with Calcein AM, bright parts show cells exhibiting green fluorescence, and the image being taken with a fluorescence microscope), and blue-purple crystals produced by NIH/3T3 cells growing in the $\alpha 1$ (I) M1 collagen hydrogel (stained with MTT, dark parts show the crystals, and the image being taken with a brightfield microscope).

FIG. 17 shows results of adherent growth of NIH/3T3 cells in $\alpha 1$ (II) and $\alpha 1$ (II) M6 collagen hydrogels,

where the three upper images show the following from left to right, respectively: NIH/3T3 cells growing adherently on the $\alpha 1$ (II) collagen hydrogel (the image being taken with a brightfield microscope), NIH/3T3 cells growing adherently on the $\alpha 1$ (II) collagen hydrogel (stained with Calcein AM, bright parts show cells exhibiting green fluorescence, and the image being taken with a fluorescence microscope), and blue-purple crystals produced by NIH/3T3 cells growing in the $\alpha 1$ (II) collagen hydrogel (stained with MTT, dark parts show the crystals, and the image being taken with a brightfield microscope); and

the three lower images show the following from left to right, respectively: NIH/3T3 cells growing adherently on the $\alpha 1$ (II) M6 collagen hydrogel (the image being taken with a brightfield microscope), NIH/3T3 cells growing adherently on the $\alpha 1$ (II) M6 collagen hydrogel (stained with Calcein AM, bright parts show cells exhibiting green fluorescence, and the image being taken with a fluorescence microscope), and blue-purple crystals produced by NIH/3T3 cells growing in the $\alpha 1$ (II) M6 collagen hydrogel (stained with MTT, dark parts show the crystals, and the image being taken with a brightfield microscope).

DETAILED DESCRIPTION OF THE EMBODIMENTS

In order to make those skilled in the art better understand the technical solutions of the present disclosure, the preferred examples of the present disclosure are described in detail below, but the following examples do not limit the protection scope of the present disclosure.

In the examples of the present disclosure, those not described in detail are all implemented

acids in the amino acid sequence remained unchanged. A homology of $\alpha 1$ (I) M1 with $\alpha 1$ (I) was 99.6%.

The mutated amino acid sequence ($\alpha 1$ (I) M1) has a full length of 1,057 AA, and is set forth in SEQ ID NO: 2.

SEQ ID NO: 2:

QLSYGYDEKSTGGISVPGPMGPSGRPLPGPPGAPGPQGFQGGPPGEPGEPGASGPPG
PPGPPGPPGKNGDDGEAGKPGRPGERGPPGPQGARGLPGTAGLPGMKGHRGFSGLDGA
KGDAGPAGPKGEPGSPGENGAPGQPGPPGLPGERGRPGAPGPAGARGNDGATGAAGPP
GPTGPAGPPGFPFPAVGAKGEAGPQGPRGSEGPQGVREPGPPGPAGAAGPAGNPGADG
QPGAKGANGAPGIAGAPGFPGARGPSGPQGGPPGPKGNSGEPGAPGSKGDTGAKGE
PGPVGVQGGPPPAGEEGKRGARGEPTGLPGPPGERGGPGSRGFPADGVAGPKGPAG
ERGSPGPAGPKGSPGEAGRPGEAGLPAGAKGLTGSPGSPGDGKTGPPGPAGQDGRPGPPG
PPGARGQAGVMGFPGPKGAAGEPGKAGERGVPPGAVGPAGKDGEAGAQQPPGPAGP
AGERGEQGPAGSPGFQGLPGPAGPPGEAGKPGEQGVPGDLGAPGPSGARGERGFPGERG
VQGGPPGPAGPRGANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGERGAAGLPGPKG
DRGDAGPKGADGSPGKDGVRGLTGPIGPPGPAGAPGDKGESGPSGPAGPTGARGAPGDR
GEPGPPGPAGFAGPPGADGQPGAKGEPGDAGAKGDAGPPGPAGPAGPPGPIGNVGAPGA
KGARGSAAGPPGATGFPGAAGRVPGPSGNAGPPGPPGPAGKEGGKGPRGETGPAGRPG
EVGPPGPPGPAGEKGSPPGADGPAGAPGTGPQGIAGQRGVVGLPGQRGERGFPLPGPS
GEPGKQGPSGASGERGPPGPMGPPGLAGPPGESGREGAPGAEGSPGRDGSPGAKGDRGE
TGPAGPPGAPGAPGAPGPVGPAGKSGDRGETGPAGPAGPVGPVGARGPAGPQGPRGDK
GETGEQGDRIKGHRGFSGLQGPPGPPGSPGEQGPSGASGPAGPRGPPGSAGAPGKDGL
NGLPGPIGPPGPRGRTGDAGPVGPPGPPGPPGPPGPPSAGFDFSFLPQPPQEKAHDGGYYY
RA.

Differences between the amino acid sequences of $\alpha 1$ (I) M1 and $\alpha 1$ (I) are shown by amino acids in bold and gray background in FIG. 1.

A DNA sequence of a gene (denoted as COL1A1M1) encoding the $\alpha 1$ (I) M1 set forth in SEQ ID NO: 2 is set forth in SEQ ID NO: 3.

SEQ ID NO: 3:

CAACTTAGTTATGGATACGATGAAAAATCCACAGGTGGAATCAGTGTTCTTGGAC
CTATGGGTCCATCAGGTCCAAGAGGTTTACCAGGACCTCCAGGTGCCCCAGGTCCCC
AGGGATTTCAAGGTCCACCAGGAGAGCCTGGTGAGCCAGGAGCTTCTGGTCCACCT
GGTCCCCCTGGACCACCTGGTCTCTCCAGGAAAGAATGGAGATGATGGTGAAGCTGGA
AAACCTGGAAGACCTGGAGAAAGAGGACCACCAGGACCCCAGGGTGCCAGAGGAC
TGCCAGGTACCGCAGGTCTGCCTGGAATGAAAGGTCATAGAGGATTTTCAGGATTAG
ACGGTGCAAAGGGAGACGCTGGACCTGCAGGACCAAAGGGTGAGCCAGGAAGTCC

AGGAGAGAATGGTGCACCAGGACAGCCAGGTCCACCTGGACTGCCCCGGTGAAAGAG
GTAGACCCGGAGCACCAGGACCAGCAGGTGCAAGAGGAAATGATGGAGCTACAGGT
GCTGCAGGACCCCCAGGTCCAACAGGACCAGCCGGTCTCCCGGTTTCCCAGGTGCC
GTTGGAGCAAAAGGTGAAGCTGGTCCACAGGGTCCAAGAGGTTCTGAAGGTCCACA
GGGAGTTAGAGGAGAACCAGGACCCCCTGGACCAGCTGGTGCAGCAGGACCAGCTG
GTAACCCTGGTGCTGACGGTCAGCCAGGTGCTAAGGGAGCAAATGGAGCACCAGGA
ATAGCTGGTGCCCCAGGATTTCCCGGTGCTAGAGGTCCAAGTGGTCCACAAGGACCA
GGAGGTCCACCCGGTCCCAAAGGAAACAGTGGAGAACCAGGTGCACCCGGTTCAAA
GGGAGATACAGGAGCTAAAGGAGAGCCCGGTCCAGTGGGTGTTTCAGGGACCACCCG
GACCTGCTGGAGAGGAAGGTAAAAGAGGTGCAAGAGGTGAGCCAGGACCAACAGG
TCTGCCTGGTCCCCCTGGTGAAAGAGGTGGTCCAGGTAGTAGAGGATTTCCAGGAGC
TGATGGTGTTGCAGGACCAAAGGGACCCGCAGGTGAGAGAGGATCACCCGGTCCAG
CCGGACCAAAGGATCACCAGGAGAAGCTGGTAGACCAGGAGAAGCTGGTCTGCCA
GGTGCTAAAGGATTGACAGGATCACCCGGTTCACCTGGTCCTGATGGAAAGACAGGA
CCTCCAGGTCCCGCTGGTCAGGACGGTAGACCAGGACCCCCAGGACCCCCAGGTGC
AAGAGGTCAGGCAGGTGTAATGGGTTTCCCCGGACCTAAAGGAGCAGCTGGAGAAC
CTGGTAAAGCTGGAGAGAGAGGAGTGCCTGGACCCCCTGGAGCTGTTGGTCCAGCA
GGAAAGGATGGTGAGGCAGGTGCACAAGGTCCACCTGGACCCGCTGGACCTGCAGG
TGAGAGAGGAGAGCAAGGTCCCGCAGGTTCTCCAGGTTTTTCAGGGTTTGCCAGGTC
CAGCCGGTCTCCTGGAGAGGCAGGAAAGCCAGGAGAACAAAGGAGTTCCAGGAGA
CCTGGGTGCACCAGGACCCTCTGGTGCAAGAGGAGAGAGAGGATTTCTGGAGAAA
GAGGTGTGCAGGGACCACCAGGTCCCGCCGGTCCAAGAGGAGCAAATGGAGCCCCT
GGAAATGACGGAGCTAAGGGTGACGCTGGTGCACCAGGAGCACCAGGTTCTCAAGG
TGCTCCCGGATTGCAGGGTATGCCTGGAGAGAGAGGTGCAGCTGGACTGCCAGGTCC
AAAAGGTGACAGAGGAGACGCCGGTCCTAAGGGAGCTGACGGTTCTCCTGGAAAGG
ACGGTGTGAGAGGTTTGACAGGACCAATAGGTCCACCCGGTCTCTGCTGGAGCCCCTG
GAGACAAAGGTGAATCAGGTCCTTCCGGTCCAGCCGGACCAACAGGAGCAAGAGGA
GCACCTGGAGACAGAGGAGAGCCAGGTCTCCAGGACCTGCAGGTTTCGCTGGTCC
TCCCGGAGCAGATGGACAGCCAGGAGCTAAGGGAGAACCCGGTGACGCTGGTGCTA
AGGGAGATGCAGGTCCACCAGGTCCTGCTGGTCCTGCTGGACCTCCCGGACCAATAG
GTAATGTTGGAGACCCGGAGCAAAAGGTGCCAGAGGTTCCGCAGGTCTCTCCGGA
GCAACTGGTTTTTCCAGGAGCTGCCGGAAGAGTGGGTCCACCTGGTCCTTCTGGAAAT
GCAGGACCACCAGGTCTCCTGGTCCAGCCGGAAGGAAGGTGGAAAGGGACCTAG
AGGAGAAACAGGTCCCGCAGGTAGACCCGGTGAGGTGGGTCCACCTGGTCCACCCG
GTCCAGCTGGTGAGAAAGGAAGTCCTGGAGCAGACGGACCAGCTGGTGCCCCTGGT
ACACCAGGACCCCAAGGAATAGCTGGTCAAAGAGGTGTTGTTGGTTTACCAGGTCAG

AGAGGAGAAAGAGGTTTTCCAGGATTACCAGGTCCCTCAGGTGAGCCCCGAAAAACA
GGGTCCCTCAGGAGCAAGTGGTGAAAGAGGACCACCAGGACCAATGGGACCTCCAG
GATTAGCTGGTCCACCAGGAGAATCAGGAAGAGAGGGTGCTCCTGGAGCAGAAGGT
TCACCAGGAAGAGACGGTTCACCCGGAGCCAAGGGAGACAGAGGTGAAACAGGTC
CCGCAGGTCCACCAGGAGCACCCGGAGCCCCTGGTGCTCCAGGACCTGTTCGGACCA
GCAGGAAAATCCGGTGACAGAGGTGAGACTGGACCCGCAGGTCTGCTGGTCCTGT
TGGACCAGTGGGTGCAAGAGGACCAGCAGGTCCACAAGGTCCAAGAGGTGACAAA
GGTGAGACAGGTGAGCAGGGTGACAGAGGAATTAAGGTACAGAGGATTTTCAGG
ACTGCAGGGACCACCCGGTCTCCCGGTCCCCAGGAGAGCAAGGTCCATCCGGTGC
ATCCGGTCCAGCTGGACCCAGAGGACCACCTGGTTCTGCTGGTGACACCAGGTAAAGA
TGGATTGAACGGTTTGCCTGGTCCAATAGGACCTCCTGGTCCAAGAGGAAGAACTGG
TGACGCCGGTCCCGTTCGGACCACCCGGTCCACCAGGTCCCCCAGGTCCACCCGGACC
ACCATCCGCAGGATTTGATTTCTCATTCCTTCCTCAACCTCCTCAAGAGAAAGCACAT
GATGGAGGTAGATACTATAGAGCC.

With reference to positions 182 to 1241 (PRO_0000005730) of a P02458 (<https://www.uniprot.org/uniprot/P02458>) sequence in the Uniprot database, an amino acid sequence of a human type II collagen $\alpha 1$ chain (denoted as $\alpha 1$ (II)) is an amino acid sequence of a human type II collagen $\alpha 1$ chain in a mature form, and does not include regions that would be processed and cleaved in $\alpha 1$ (II) proprotein, such as a signal peptide, a C-terminal propeptide, and an N-terminal propeptide, and is set forth in SEQ ID NO: 4.

SEQ ID NO: 4:

QMAGGFDEKAGGAQLGVMQGPMPGMPGRGPPGPAGAPGPQGFQGNPGEPPGEPGV
SGPMPGRGPPGPPGKPGDDGEAGKPGKAGERGPPGPQGARGFPGTPGLPGVKGHRGYP
GLDGAKGEAGAPGVKGESGSPGENSGSPGMPGRGLPGERGRTGPAGAAGARGNDGQP
GPAGPPGPVGPAGGPGFPGAPGAKGEAGPTGARGPEGAQGPREGPGTPGSPGPAGASGN
PGTDGIPGAKGSAGAPGIAGAPGFPGRGPPGPQGATGPLGPKGQTGEPGIAGFKGEQGP
KGEPGPAGPQGAPGPAGEEGKRGARGEPPGVPIGPPGERGAPGNRGFPQDGLAGPK
GAPGERGPSGLAGPKGANGDPGRPGEPGLPGARGLTGRPGDAGPQGVGPPSGAPGEDG
RPGPPGPQGARGQPGVMGFPGPKGANGEPGKAGEKGLPGAPGLRGLPGKDGETGAAGP
PGPAGPAGERGEQAPGPSGFQGLPGGPPGEGGKPGDQGVPGEAGAPGLVGPRGERG
FPERGSPGAQQLQGPRGLPGTPGTDGPKGASGPAGPPGAQGPGLQGMPGERGAAGIA
GPKGDRGDVGEKGPEGAPGKDGGRLTGPIGPPGPAGANGEKGEVGGPPGAGSAGARG
APGERGETGPPGPAGFAGPPGADGQPGAKGEQGEAGQKGDAGAPGPQGPSGAPGPQGP
TGVTPGPKGARGAQGPATGFPGAAGRVGPPGSNGNPGGPPGPSGKDGPKGARGDS
GPPGRAGEPGLQGPAGPPGEKGEPGDDGPSGAEGPPGPQGLAGQRGIVGLPGQRGERGF
PGLPGPSGEPGKQGAPGASGDRGPPGPVGGPGLTGPAEPGREGSPGADGPPGRDGAAG

VKGDRGETGAVGAPGAPGPPGSPGPAGPTGKQGDRGEAGAQQGPMGSPGPAGARGIQGP
QGPRGDKGEAGEPGERGLKGHRGFTGLQGLPGPPGPSGDQGASGPAGPSGPRGPPGPVG
PSGKDGANGIPGPIGPPGPRGRSGETGPAGPPGNPGPPGPPGPPGPGIDMSAFAGLGPREK
GPDPLQYMRA.

A long-term experimental research was performed to obtain a variant of a recombinant human type II collagen $\alpha 1$ chain, which was denoted as $\alpha 1$ (II) M6. Compared with the amino acid sequence of $\alpha 1$ (II), 9 amino acids were mutated to proline (Pro, abbreviated as P) in $\alpha 1$ (II) M6. That is, V at position 67, M at position 68, M at position 72, M at position 75, R at position 78, M at position 108, R at position 111, M at position 162, and R at position 165 in the amino acid sequence set forth in SEQ ID NO: 4 all were mutated to P, and the remaining amino acids in the amino acid sequence remained unchanged. A homology of $\alpha 1$ (II) M6 with $\alpha 1$ (II) was 99.2%.

The mutated amino acid sequence ($\alpha 1$ (II) M6) has a full length of 1,060 AA, and is set forth in SEQ ID NO: 5.

SEQ ID NO: 5:

QMAGGFDEKAGGAQLGPPQGPPGPPGPPGPPGPAGAPGPQGFQGNPGEPGEPGVSG
PPGPPGPPGPPGPKGDDGEAGKPKGAGERGPPGPQGARGFPGTGPLGVKGHRGYPLD
GAKGEAGAPGVKGESGSPGENSGPPGPPGLPGERGRTGPAGAAGARGNDGQPGPAG
PPGPVGPAGGPGFPGAPGAKGEAGPTGARGPEGAQQPRGEPGTPGSPGPAGASGNPGTD
GIPGAKGSAGAPGIAGAPGFPGRGPPGPQGATGPLGPKGQTGEPGIAGFKGEQGPKEP
GPAGPQGAPGPAGEEGKRGARGEPGGVGPPIGPPGERGAPGNRGFPQDGLAGPKGAPG
ERGPSGLAGPKGANGDPGRPGEPGLPGARGLTGRPGDAGPQGVGSPGAPGEDGRPGPP
GPQGARGQPGVMGFPGPKGANGEPGKAGEKGLPGAPGLRGLPGKDGETGAAGPPGPA
GPAGERGEQGAPGPSGFQGLPGPPGPPGEGGKPGDQGVPGEAGAPGLVGPRGERGFPG
RGSPGAQGLQGPRGLPGTPGTDGPKGASGPAGPPGAQQGPPGLQGMPPERGAAGIAGPK
GDRGDVGEKGPEGAPGKDGGRLTGPIGPPGPAGANGEKGEVGGPPGAGSAGARGAPG
ERGETGPPGPAGFAGPPGADGQPGAKGEQGEAGQKGDAGAPGPQGPSGAPGPQGPTGV
TGPKGARGAQGPPGATGFPGAAGRVGPPGSNGNPGGPPGPPGPSKDGPKGARGDSGPPG
RAGEPGLQGAPGPPGEKGEPGDDGPSGAEGPPGPQGLAGQRGIVGLPGQRGERGFPLP
GPSGEPGKQGAPGASGDRGPPGPVGPPGLTGPAEPGREGSPGADGPPGRDGAAGVKG
DRGETGAVGAPGAPGPPGSPGPAGPTGKQGDRGEAGAQQGPMGSPGPAGARGIQGPQGP
RGDKGEAGEPGERGLKGHRGFTGLQGLPGPPGPSGDQGASGPAGPSGPRGPPGPVGPSPG
KDGANGIPGPIGPPGPRGRSGETGPAGPPGNPGPPGPPGPPGPGIDMSAFAGLGPREKGP
DPLQYMRA.

Differences between the amino acid sequences of $\alpha 1$ (II) M6 and $\alpha 1$ (II) are shown by amino acids in bold and gray background in FIG. 2.

A DNA sequence of a gene (denoted as COL2A1M6) encoding the $\alpha 1$ (II) M6 set forth in SEQ ID NO: 5 is set forth in SEQ ID NO: 6.

SEQ ID NO: 6:

CAAATGGCTGGTGGATTCGATGAAAAGGCTGGTGGAGCCCAATTAGGTCCTCCA
CAAGGTCCTCCCGGTCCACCTGGTCCTCCCGGTCTCCAGGTCCCGCCGGTGCTCCT
GGACCACAGGGTTTCCAAGGAAACCCCGGTGAACCAGGTGAGCCTGGTGTTTCAGG
TCCTCCCGGTCTCTCCAGGACCACCTGGACCACCAGGAAAGCCTGGTGACGACGGAG
AAGCTGGTAAACCAGGAAAGGCAGGAGAGAGAGGTCCACCTGGACCTCAGGGTGCC
AGAGGTTTCCCAGGTACCCCTGGTCTTCTGGTGTCAAGGGTCATAGAGGTTACCCC
GGTTTGGATGGTGCCAAGGGTGAAGCCGGTGCCCTGGTGTTAAGGGTGAATCAGGA
AGTCCCGGTGAAAATGGAAGTCCCGGTCCACCCGGTCCACCTGGACTGCCAGGTGA
GAGAGGAAGAACCGGACCAGCTGGTGCTGCAGGTGCTAGAGGAAATGACGGACAGC
CCGGACCAGCCGGACCTCCCGGTCTGTTGGGCCCGCAGGTGGTCCTGGTTTCCCtgG
TGCTCCTGGAGCCAAAGGAGAAGCCGGACCCACCGGAGCCAGAGGTCCCGAGGGA
GCACAGGGACCTAGAGGAGAACCAGGTACACCAGGTAGTCCCGGTCTCTGCTGGTGC
ATCAGGAAATCCCGGAACTGACGGTATTCCAGGAGCAAAGGGATCTGCAGGAGCACC
AGGAATAGCTGGTGCTCCTGGATTTCCAGGTCCCAGAGGACCTCCCGGTCTCAAGG
AGCAACAGGTCCTTTGGGACCAAAAGGTCAAACAGGAGAACCAGGTATTGCTGGAT
TCAAAGGAGAGCAAGGTCCAAAGGGAGAGCCCGGTCCCGCAGGTCCCCAAGGAGC
CCCAGGACCAGCTGGTGAAGAAGGAAAAAGAGGAGCCAGAGGTGAACCTGGAGGA
GTAGGACCTATTGGTCCTCCTGGTGAGAGAGGTGCTCCCGGAAACAGAGGTTTTCT
GGTCAAGATGGTCTGGCTGGACCTAAAGGTGCTCCAGGAGAGAGAGGACCTTCAGG
ACTTGCTGGTCCAAAAGGTGCTAACGGAGATCCAGGAAGACCCGGTGAACCTGGTCT
GCCTGGAGCTAGAGGATTAACAGGAAGACCAGGTGACGCAGGTCCCCAGGGTAAAG
TGGGTCCCAGTGGTGCCCCAGGTGAAGATGGAAGACCTGGTCCTCCCGGACCCCAA
GGTGCAAGAGGTCAGCCTGGAGTGATGGGATTTCTGGACCCAAGGGTGCTAACGG
AGAACCTGGAAAAGCTGGTGAGAAAGGACTGCCCGGTGCCCCAGGTCTTAGAGGTT
TGCCAGGTAAAGATGGAGAAACAGGAGCCGCAGGACCACCCGGTCCAGCCGGACCA
GCAGGAGAGAGAGGTGAACAAGGAGCACCTGGTCCAAGTGGTTTTTCAGGGTCTTCC
AGGTCCCCCTGGTCCACCAGGAGAGGGAGGTAAACCAGGTGACCAAGGTGTCCCTG
GAGAAGCAGGTGCACCCGGTCTTGTGGGTCCAAGAGGTGAAAGAGGATTCCCTGGT
GAGAGAGGATCTCCCGGAGCCCAGGGACTTCAAGGTCTTAGAGGTCTGCCAGGTAC
CCCTGGTACAGACGGACCAAAAGGGAGCATCAGGACCCGCTGGACCTCCCGGAGCCC
AAGGTCCTCCAGGTTTACAAGGTATGCCTGGTGAAAGAGGTGCTGCAGGTATAGCTG
GACCAAAAGGAGACAGAGGTGACGTTGGTGAGAAGGGTCCCGAAGGAGCCCCTGG
AAAAGATGGTGGAAGAGGATTAACAGGTCCTATAGGACCACCCGGTCCAGCCGGTGC

TAATGGAGAAAAAGGAGAAGTAGGTCTCCAGGTCCAGCAGGATCTGCAGGTGCTA
GAGGTGCCCCCTGGAGAGAGAGGTGAAACAGGACCACCTGGTCCAGCTGGTTTCGCT
GGTCCCCCAGGAGCTGATGGACAGCCCGGTGCAAAAGGTGAACAAGGAGAAGCCG
GACAGAAGGGAGATGCTGGAGCCCCCGGTCCACAAGGTCCCTCAGGAGCACCAGGT
CCTCAAGGTCCAACCTGGTGTGACCGGGCCAAAGGGTGCAAGAGGAGCACAGGGACC
TCCAGGAGCAACAGGTTTCCCAGGAGCTGCTGGTAGAGTCGGTCCACCCGGATCTAA
TGGTAACCCCGGACCACCAGGACCACCTGGACCATCTGGAAAGGATGGACCCAAAG
GAGCAAGAGGAGATTTCAGGACCACCCGGAAGAGCAGGAGAACCTGGATTACAGGGT
CCCGCCGGTCCACCAGGAGAGAAAGGAGAGCCCGGAGATGATGGTCCCTCAGGTGC
AGAGGGACCCCCAGGACCCCAAGGTCTGGCAGGTCAAAGAGGTATAGTGGGTCTTC
CAGGTCAAAGAGGTGAAAGAGGATTTCCAGGACTTCCAGGTCCTTCAGGTGAACCC
GGTAAACAGGGAGCCCCCGGAGCCTCAGGTGACAGAGGTCCCTCCAGGACCAGTAGG
ACCCCCAGGTTTAACCGGACCAGCAGGTGAGCCAGGAAGAGAAGGTTCTCCTGGAG
CCGATGGACCTCCAGGAAGAGACGGTGCAGCTGGTGTAAAGGGTGACAGAGGTGAA
ACTGGAGCCGTAGGAGCCCCAGGTGCCCCCGGACCACCCGGATCACCCGGACCTGC
AGGTCCTACTGGTAAACAAGGAGATAGAGGAGAAGCCGGTGCCCAGGGTCCTATGG
GTCCTTCTGGTCCTGCAGGAGCAAGAGGTATACAAGGTCCACAGGGTCCCAGAGGTG
ACAAGGGTGAAGCAGGAGAACCCGGTGAGAGAGGTCTGAAGGGTCATAGAGGATTC
ACCGGGTTACAGGGTTTGCCAGGACCCCCCTGGACCAAGTGGTGACCAGGGTGCATCC
GGTCCAGCAGGTCCCTTCTGGACCAAGAGGTCCCTCCCGGTCCAGTTGGTCCATCAGGT
AAAGACGGAGCCAACGGTATCCCAGGTCCCATCGGTCCTCCAGGTCCTAGAGGAAGA
AGTGGAGAGACTGGTCCTGCTGGACCTCCTGGAAACCCTGGTCCTCCAGGACCTCCA
GGTCCTCCAGGTCCCGGAATAGATATGTCCGCTTTCGCTGGATTGGGACCAAGAGAG
AAAGGTCCTGACCCTCTTCAATATATGAGAGCA.

Two termini of the DNA sequence encoding the $\alpha 1$ (I) M1 were modified as follows: a DNA sequence encoding a Strep-Tag II tag was added to the amino terminus and a DNA sequence encoding a 6 $\times$ His Tag was added to the carboxyl terminus; and $\alpha 1$ (I) M1 protein with the tags was finally obtained by expression, which has 1,071 amino acids in total and is set forth in SEQ ID NO: 7.

SEQ ID NO: 7:

WSHPQFEKQLSYGYDEKSTGGISVPGPMGPSGRGLPGPPGAPGPQGFQGPPEPG
EPGASGPPGPPGPPGKNGDDGEAGKPGRPGERGPPGPQGARGLPGTAGLPGMKGH
RGFSGLDGAKGDAGPAGPKGEPGSPGENGAPGQPGLPGERGRPGAPGPAGARGND
GATGAAGPPGPTGPAGPPGFPGAVGAKGEAGPQGPRGSEGPQGVRRGEPGPPGPAGAAGP
AGNPGADGQPGAKGANGAPGIAGAPGFPGARGPSGPQGPGGPPGPKNSGEPGAPGSK
GDTGAKGEPGPVGVQGPMPAGEEGKRGARGEPGPTGLPGPPGERGGPGSRGFPADGV

AGPKGPAGERGSPGPAGPKGSPGEAGRPGEAGLPGAKGLTGSPGSPGPDGKTGPPGPAG
 QDGRPGPPGPPGARGQAGVMGFPGPKGAAGEPGKAGERGVPGPPGAVGPAGKDGEAG
 AQGPPGPAGPAGERGEQGPAGSPGFQGLPGPAGPPGEAGKPGEQGVPGDLGAPGPSGAR
 GERGFPGERGVQPPGPAGPRGANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPPER
 GAAGLPGPKGDRGDAGPKGADGSPGKDGVRLTGPIGPPGPAGAPGDKGESGPSGPAGP
 TGARGAPGDRGEPGPPGAGFAGPPGADGQPGAKGEPGDAGAKGDAGPPGPAGPAGPP
 GPIGNVGAPGAKGARGSAAGPPGATGFPAAAGRVGPPGPSGNAGPPGPPGPAGKEGGKGP
 RGETGPAGRPGEVGP GPPGPAGEKGSPGADGPAGAPGTPGPQGIAGQRGVVGLPGQRG
 ERGFPLPGPSGEPGKQGPSGASGERGPPGPMGPPGLAGPPGESGREGAPGAEGSPGRD
 GSPGAKGDRGETGPAGPPGAPGAPGAPGVPVGPAGKSGDRGETGPAGPAGVPVGPARGP
 AGPQGPRGDKGETGEQGDRGIKGHRGFSGLQGPPGPPGSPGEQGPSGASGPAGPRGPPG
 SAGAPGKDGLNGLPGPIGPPGPRGRTGDAGVPVGP GPPGPPGPPGPPSAGFDFSFLPQPPQ
 EKAHDGGRYYRAHHHHHH.

After optimization design, a DNA sequence of a gene (denoted as COL1A1M1) encoding the amino acid sequence ($\alpha 1$ (I) M1) set forth in SEQ ID NO: 7 is set forth in SEQ ID NO: 8.

SEQ ID NO: 8:

TGGTCTCATCCACAATTTGAAAAGCAACTTAGTTATGGATACGATGAAAAATCCA
 CAGGTGGAATCAGTGTTCTTGGACCTATGGGTCCATCAGGTCCAAGAGGTTTACCAG
 GACCTCCAGGTGCCCCAGGTCCCCAGGGATTTCAAGGTCCACCAGGAGAGCCTGGT
 GAGCCAGGAGCTTCTGGTCCACCTGGTCCCCCTGGACCACCTGGTCCTCCAGGAAAG
 AATGGAGATGATGGTGAAGCTGGAAAACCTGGAAGACCTGGAGAAAGAGGACCACC
 AGGACCCCAGGGTGCCAGAGGACTGCCAGGTACCGCAGGTCTGCCTGGAATGAAAG
 GTCATAGAGGATTTTCAGGATTAGACGGTGCAAAGGGAGACGCTGGACCTGCAGGAC
 CAAAGGGTGAGCCAGGAAGTCCAGGAGAGAATGGTGCACCAGGACAGCCAGGTCC
 ACCTGGACTGCCCCGGTGAAAGAGGTAGACCCGGAGCACCAGGACCAGCAGGTGCAA
 GAGGAAATGATGGAGCTACAGGTGCTGCAGGACCCCCAGGTCCAACAGGACCAGCC
 GGTCTCCCGGTTTCCCAGGTGCCGTTGGAGCAAAAGGTGAAGCTGGTCCACAGGG
 TCCAAGAGGTTCTGAAGGTCCACAGGGAGTTAGAGGAGAACCAGGACCCCCTGGAC
 CAGCTGGTGCAGCAGGACCAGCTGGTAACCCTGGTGCTGACGGTCAGCCAGGTGCT
 AAGGGAGCAAATGGAGCACCAGGAATAGCTGGTGCCCCAGGATTTCCCGGTGCTAGA
 GGTCCAAGTGGTCCACAAGGACCAGGAGGTCCACCCGGTCCCAAAGGAAACAGTGG
 AGAACCAGGTGCACCCGGTTCAAAGGGAGATACAGGAGCTAAAGGAGAGCCCGGTC
 CAGTGGGTGTTTCAGGGACCACCCGGACCTGCTGGAGAGGAAGGTAAAAGAGGTGCA
 AGAGGTGAGCCAGGACCAACAGGTCTGCCTGGTCCCCCTGGTGAAAGAGGTGGTCC
 AGGTAGTAGAGGATTTCCAGGAGCTGATGGTGTTGCAGGACCAAAGGGACCCGCAG
 GTGAGAGAGGATCACCCGGTCCAGCCGGACCAAAAGGATCACCAGGAGAAGCTGGT

AGACCAGGAGAAGCTGGTCTGCCAGGTGCTAAAGGATTGACAGGATCACCCGGTTC
ACCTGGTCCTGATGGAAAGACAGGACCTCCAGGTCCCGCTGGTCAGGACGGTAGAC
CAGGACCCCCAGGACCCCCAGGTGCAAGAGGTCAGGCAGGTGTAATGGGTTTCCCC
GGACCTAAAGGAGCAGCTGGAGAACCTGGTAAAGCTGGAGAGAGAGGAGTGCCTG
GACCCCTGGAGCTGTTGGTCCAGCAGGAAAGGATGGTGAGGCAGGTGCACAAGGT
CCACCTGGACCCGCTGGACCTGCAGGTGAGAGAGGAGAGCAAGGTCCCGCAGGTTC
TCCAGGTTTTTCAGGGTTTGCCAGGTCCAGCCGGTCCTCCTGGAGAGGCAGGAAAGCC
AGGAGAACAAGGAGTTCCAGGAGACCTGGGTGCACCAGGACCCTCTGGTGCAAGAG
GAGAGAGAGGATTTCTGGAGAAAGAGGTGTGCAGGGACCACCAGGTCCCGCCGGT
CCAAGAGGAGCAAATGGAGCCCCTGGAAATGACGGAGCTAAGGGTGACGCTGGTGC
ACCAGGAGCACCAGGTTCTCAAGGTGCTCCCGGATTGCAGGGTATGCCTGGAGAGAG
AGGTGCAGCTGGACTGCCAGGTCCAAAAGGTGACAGAGGAGACGCCGGTCCTAAGG
GAGCTGACGGTTCTCCTGGAAAGGACGGTGTGAGAGGTTTGACAGGACCAATAGGT
CCACCCGGTCCTGCTGGAGCCCCTGGAGACAAAGGTGAATCAGGTCCTTCCGGTCCA
GCCGGACCAACAGGAGCAAGAGGAGCACCTGGAGACAGAGGAGAGCCAGGTCTCTC
CAGGACCTGCAGGTTTCGCTGGTCCTCCCGGAGCAGATGGACAGCCAGGAGCTAAG
GGAGAACCCGGTGACGCTGGTGCTAAGGGAGATGCAGGTCCACCAGGTCTGCTGG
TCCTGCTGGACCTCCCGGACCAATAGGTAATGTTGGAGCACCCGGAGCAAAAGGTGC
CAGAGGTTCCGCAGGTCTCTCCCGGAGCAACTGGTTTTCCAGGAGCTGCCGGAAGAG
TGGGTCCACCTGGTCCTTCTGGAAATGCAGGACCACCAGGTCTCCTGGTCCAGCCG
GAAAGGAAGGTGGAAAGGGACCTAGAGGAGAAACAGGTCCCGCAGGTAGACCCGG
TGAGGTGGGTCCACCTGGTCCACCCGGTCCAGCTGGTGAGAAAGGAAGTCCTGGAG
CAGACGGACCAGCTGGTGCCCCTGGTACACCAGGACCCCAAGGAATAGCTGGTCAA
AGAGGTGTTGTTGGTTTACCAGGTCAGAGAGGAGAAAGAGGTTTTCCAGGATTACCA
GGTCCCTCAGGTGAGCCCGGAAAAACAGGGTCCCTCAGGAGCAAGTGGTGAAAGAGG
ACCACCAGGACCAATGGGACCTCCAGGATTAGCTGGTCCACCAGGAGAATCAGGAA
GAGAGGGTGCTCCTGGAGCAGAAGGTTACCAGGAAGAGACGGTTCACCCGGAGCC
AAGGGAGACAGAGGTGAAACAGGTCCCGCAGGTCCACCAGGAGCACCCGGAGCCC
CTGGTGCTCCAGGACCTGTCGGACCAGCAGGAAAATCCGGTGACAGAGGTGAGACT
GGACCCGCAGGTCTGCTGGTCCTGTTGGACCAGTGGGTGCAAGAGGACCAGCAGG
TCCACAAGGTCCAAGAGGTGACAAAGGTGAGACAGGTGAGCAGGGTGACAGAGGA
ATTAAAGGTCACAGAGGATTTTCAGGACTGCAGGGACCACCCGGTCTCCCGGTTCC
CCAGGAGAGCAAGGTCCATCCGGTGCATCCGGTCCAGCTGGACCCAGAGGACCACC
TGGTTCTGCTGGTGCACCAGGTAAAGATGGATTGAACGGTTTGCTGGTCCAATAGG
ACCTCCTGGTCCAAGAGGAAGAACTGGTGACGCCGGTCCCGTCGGACCACCCGGTC
CACCAGGTCCCCCAGGTCCACCCGGACCACCATCCGCAGGATTTGATTTCTCATTCCT

TCCTCAACCTCCTCAAGAGAAAGCACATGATGGAGGTAGATACTATAGAGCCCATCAC
CACCATCATCATTA.

Two termini of the DNA sequence encoding the $\alpha 1$ (II) M6 were modified as follows: a DNA sequence encoding a Strep-Tag II tag was added to the amino terminus and a DNA sequence encoding a 6 $\times$ His Tag was added to the carboxyl terminus; and $\alpha 1$ (II) M6 protein with the tags was finally obtained by expression, which has 1,076 amino acids in total and is set forth in SEQ ID NO: 9.

SEQ ID NO: 9:

EFWSHPQFEKQ MAGGFDEKAGGAQLGPPQGPPGPPGPPGPPGAPGPQGFQGNP
GEPGEPGVSGPPGPPGPPGPPGPKPDDGEAGKPGKAGERGPPGPQGARGFPPTPLPGV
KGHRGYPLDGAKEAGAPGVKGESGSPGENGSPGPPGPPGLPGERGRTGPAGAAGAR
GNDGQPGPAGPPGPVGPAGGPGFPGAPGAKGEAGPTGARGPEGAQGPARGPTPGSPGP
AGASGNPGTDGIPGAKGSAGAPGIAGAPGFPGRGPPGPQGATGPLGPKGQTGEPGIAGF
KGEQGPKGEPGPAGPQGPAGEEGKRGARGEPGGVGPPIGPPGERGAPGNRGFPQD
GLAGPKGAPGERGPSGLAGPKGANGDPGRPGEPGLPGARGLTGRPGDAGPQGVGPSG
APGEDGRPGPPGPQARGQPGVMGFPGPKGANGEPGKAGEKGLPGAPGLRGLPGKDGE
TGAAGPPGPAGPAGERGEQGAPGPSGFQGLPGPPGPPGEGGKPGDQGVPEAGAPGLVG
PRGERGFPGERGSPGAQGLQGPRGLPGTPGTDGPKGASGPAGPPGAQGPGLQGMPPER
GAAGIAGPKGDRGDVGEKGPEGAPGKDGGRLTGPIGPPGPAGANGEKGEVGPMPGAPS
AGARGAPGERGETGPPGPAGFAGPPGADGQPGAKGEQGEAGQKGDAGAPGPQGPSGAP
GPQGPTGVTGPKGARGAQGPAGATGFPGAAGRVPGPSNGNP GPPGPPGPSGKDGPKG
ARGDSGPPGRAGEPGLQGPAGPPGEKGEPGDDGPSGAEGPPGPQGLAGQRGIVGLPGQR
GERGFPGLPGSPGEPGKQGAPGASGDRGPPGPVGPPLTGPAEPGREGSPGADGPPGR
DGAAGVKGDRGETGAVGAPGAPPPGSPGPAGPTGKQGDRGEAGAQGPMGPSGPAGA
RGIQGPQGPGRGDKGEAGEPGERGLKGHRGFTGLQGLPGPPGPSGDQGASGPAGPSGPRG
PPGPVGPSGKD GANGIPGPIGPPGPRGRSGETGPAGPPGNPGPPGPPGPPGIDMSAFAG
LGPREKGPDP LQYMR AHHHHH.

After optimization design, a DNA sequence of a gene (denoted as COL2A1M6) encoding the amino acid sequence ($\alpha 1$ (II) M6) set forth in SEQ ID NO: 9 is set forth in SEQ ID NO: 10.

SEQ ID NO: 10:

GAATTCTGGAGTCATCCTCAATTCGAAAAACAAATGGCTGGTGGATTCGATGAA
AAGGCTGGTGGAGCCCAATTAGGTCCTCCACAAGGTCCTCCCGGTCCACCTGGTCCT
CCCGGTCTCCAGGTCCCGCCGGTGCTCCTGGACCACAGGGTTTCCAAGGAAACCCC
GGTGAACCAGGTGAGCCTGGTGTTTCAGGTCCTCCCGGTCTCCAGGACCACCTGGA
CCACCAGGAAAGCCTGGTGACGACGGAGAAGCTGGTAAACCAGGAAAGGCAGGAG
AGAGAGGTCCACCTGGACCTCAGGGTGCCAGAGGTTTCCAGGTACCCCTGGTCTTC

CTGGTGTCAAGGGTCATAGAGGTTACCCCGGTTTGGATGGTGCCAAGGGTGAAGCCG
GTGCCCCTGGTGTTAAGGGTGAATCAGGAAGTCCCGGTGAAAATGGAAGTCCCGGTC
CACCCGGTCCACCTGGACTGCCAGGTGAGAGAGGAAGAACCGGACCAGCTGGTGCT
GCAGGTGCTAGAGGAAATGACGGACAGCCCGGACCAGCCGGACCTCCCGGTCCTGT
TGGGCCCCGCAGGTGGTCCTGGTTTCCCtgGTGCTCCTGGAGCCAAAGGAGAAGCCGG
ACCCACCGGAGCCAGAGGTCCCGAGGGAGCACAGGGACCTAGAGGAGAACCAGGT
ACACCAGGTAGTCCCGGTCCTGCTGGTGCATCAGGAAATCCCGGAACTGACGGTATT
CCAGGAGCAAAGGGATCTGCAGGAGCACCAGGAATAGCTGGTGCTCCTGGATTTC
GGTCCCAGAGGACCTCCCGGTCCTCAAGGAGCAACAGGTCCCTTGGGACCAAAAGG
TCAAACAGGAGAACCAGGTATTGCTGGATTCAAAGGAGAGCAAGGTCCAAAGGGAG
AGCCCGGTCCCGCAGGTCCCCAAGGAGCCCCAGGACCAGCTGGTGAAGAAGGAAA
AAGAGGAGCCAGAGGTGAACCTGGAGGAGTAGGACCTATTGGTCCTCCTGGTGAGA
GAGGTGCTCCCGGAAACAGAGGTTTTCTGGTCAAGATGGTCTGGCTGGACCTAAAG
GTGCTCCAGGAGAGAGAGGACCTTCAGGACTTGCTGGTCCAAAAGGTGCTAACGGA
GATCCAGGAAGACCCGGTGAACCTGGTCTGCCTGGAGCTAGAGGATTAACAGGAAG
ACCAGGTGACGCAGGTCCCCAGGGTAAAGTGGGTCCCAGTGGTGCCCCAGGTGAAG
ATGGAAGACCTGGTCCTCCCGGACCCCAAGGTGCAAGAGGTCAGCCTGGAGTGATG
GGATTTCTGGACCCAAGGGTGCTAACGGAGAACCTGGAAAAGCTGGTGAGAAAGG
ACTGCCCGGTGCCCCAGGTCTTAGAGGTTTGCCAGGTAAAGATGGAGAAACAGGAG
CCGCAGGACCACCCGGTCCAGCCGGACCAGCAGGAGAGAGAGGTGAACAAGGAGC
ACCTGGTCCAAGTGGTTTTTCAGGGTCTTCCAGGTCCCCCTGGTCCACCAGGAGAGGG
AGGTAAACCAGGTGACCAAGGTGTCCCTGGAGAAGCAGGTGCACCCGGTCTTGTGG
GTCCAAGAGGTGAAAGAGGATTCCCTGGTGAGAGAGGATCTCCCGGAGCCCAGGGA
CTTCAAGGTCTTAGAGGTCTGCCAGGTACCCCTGGTACAGACGGACCAAGGGAGCA
TCAGGACCCGCTGGACCTCCCGGAGCCCAAGGTCCCTCCAGGTTTACAAGGTATGCCT
GGTGAAAGAGGTGCTGCAGGTATAGCTGGACCAAAAGGAGACAGAGGTGACGTTGG
TGAGAAGGGTCCCGAAGGAGCCCCCTGGAAAAGATGGTGGAAGAGGATTAACAGGTC
CTATAGGACCACCCGGTCCAGCCGGTGCTAATGGAGAAAAAGGAGAAGTAGGTCCTC
CAGGTCCAGCAGGATCTGCAGGTGCTAGAGGTGCCCCTGGAGAGAGAGGTGAAACA
GGACCACCTGGTCCAGCTGGTTTCGCTGGTCCCCCAGGAGCTGATGGACAGCCCGGT
GCAAAAGGTGAACAAGGAGAAGCCGGACAGAAGGGAGATGCTGGAGCCCCCGGTC
CACAAGGTCCCTCAGGAGCACCAGGTCTCAAGGTCCAACCTGGTGTGACCGGGCCA
AAGGGTGCAAGAGGAGCACAGGGACCTCCAGGAGCAACAGGTTTCCCAGGAGCTG
CTGGTAGAGTCGGTCCACCCGGATCTAATGGTAACCCCGGACCACCAGGACCACCTG
GACCATCTGGAAAGGATGGACCCAAAGGAGCAAGAGGAGATTCAGGACCACCCGGA
AGAGCAGGAGAACCTGGATTACAGGGTCCCGCCGGTCCACCAGGAGAGAAAGGAGA

GCCCGGAGATGATGGTCCCTCAGGTGCAGAGGGACCCCCAGGACCCCAAGGTCTGG
CAGGTCAAAGAGGTATAGTGGGTCTTCCAGGTCAAAGAGGTGAAAGAGGATTTCCA
GGACTTCCAGGTCCTTCAGGTGAACCCGGTAAACAGGGAGCCCCCGGAGCCTCAGG
TGACAGAGGTCCTCCAGGACCAGTAGGACCCCCAGGTTTAACCGGACCAGCAGGTG
AGCCAGGAAGAGAAGGTTCTCCTGGAGCCGATGGACCTCCAGGAAGAGACGGTGCA
GCTGGTGTAAAGGGTGACAGAGGTGAACTGGAGCCGTAGGAGCCCCAGGTGCCCC
CGGACCACCCGGATCACCCGGACCTGCAGGTCCTACTGGTAAACAAGGAGATAGAG
GAGAAGCCGGTGCCAGGGTCCTATGGGTCTTCTGGTCCTGCAGGAGCAAGAGGTA
TACAAGGTCCACAGGGTCCCAGAGGTGACAAGGGTGAAGCAGGAGAACCCGGTGA
GAGAGGTCTGAAGGGTCATAGAGGATTCACCGGGTTACAGGGTTTGCCAGGACCCCC
TGGACCAAGTGGTGACCAGGGTGCATCCGGTCCAGCAGGTCCTTCTGGACCAAGAG
GTCCTCCCGGTCCAGTTGGTCCATCAGGTAAAGACGGAGCCAACGGTATCCAGGTC
CCATCGGTCCTCCAGGTCCTAGAGGAAGAAGTGGAGAGACTGGTCCTGCTGGACCTC
CTGGAAACCCTGGTCCTCCAGGACCTCCAGGTCCTCCAGGTCCCGGAATAGATATGTC
CGCTTTCGCTGGATTGGGACCAAGAGAGAAAGGTCCTGACCCTCTTCAATATATGAG
AGCACACCATCACCATCATCACTAA.

The synthesis of DNA sequences was entrusted to Nanjing Genscript Biotechnology Co., Ltd. to synthesize DNA fragments of the genes set forth in SEQ ID NO: 8 and SEQ ID NO: 10.

Example 2 Construction of recombinant expression vectors and screening of strains

(1) Construction of recombinant expression vectors

Synthesized gene fragments set forth in SEQ ID NO: 8 and SEQ ID NO: 10 were recombined into a pPIC9K empty vector (purchased from Thermo Fisher Scientific) with a target fragment being accurately inserted into a secretion signal α factor-containing reading frame of a secretory vector to obtain a recombinant expression vector plasmid pPIC9K-COL2A1M6 expressing $\alpha 1$ (II) M6 and a recombinant expression vector plasmid pPIC9K-COL1A1M1 expressing $\alpha 1$ (I) M1.

The plasmids pPIC9K-COL2A1M6 and pPIC9K-COL1A1M1 were transformed into competent *Escherichia coli* DH5 α (purchased from the Sangon Biotech (Shanghai) Co., Ltd.), positive clones were screened on an LB resistant plate with ampicillin, and the recombinant plasmids were extracted for sequencing (which was entrusted to the Sangon Biotech (Shanghai) Co., Ltd.). Sequencing results showed that the recombinant plasmids were correct. Maps of the plasmids pPIC9K-COL1A1M1 and pPIC9K-COL2A1M6 are shown in FIG. 3 and FIG. 4, respectively.

(2) Screening of strains

10 μ g of the recombinant expression vector plasmids was digested overnight at 37°C with SacI (SacI was purchased from the Dalian TaKaRa, and specific operations were performed

according to instructions of a kit) for linearization, and then a PCR product purification kit (purchased from the Sangon Biotech (Shanghai) Co., Ltd.) was used to recover a linearized plasmid, with a volume controlled at about 10 μ L.

The linearized plasmid was electrotransformed into a host *Pichia pastoris* SMD1168 (purchased from Thermo Fisher Scientific) competent cell, an electrotransformed *Pichia pastoris* solution was coated on MD plates with 100 μ L to 200 μ L for each plate, and the plates were allowed to stand at room temperature for 10 min and invertedly incubated at 30°C for 2 d to 5 d until single colonies (positive transformants) appeared.

2 mL of sterile double-distilled water was added to a surface of each MD plate, then His⁺ transformants on the surface of the plate were gently scraped off with a sterile triangular spreader, transferred to a 50 mL centrifuge tube, and diluted with sterile double-distilled water to obtain a suspension, 10⁵ cells were coated on a YPD plate including 0.5 mg/mL G418, and the plate was inverted and incubated at 30°C for 3 d to 4 d until single colonies appeared. Colonies were picked from the YPD plate and added to a first sterile 96-well plate (200 μ L of YPD/well), and a resulting mixture in each well was thoroughly mixed and incubated at 30°C for 48 h to obtain a first cultivation system. The first cultivation system in each well of the first sterile 96-well plate was thoroughly mixed, and 10 μ L of the first cultivation system was taken, added to a second sterile 96-well plate, and then incubated at 30°C for 24 h to obtain a second cultivation system. The second cultivation system in each well of the second sterile 96-well plate was thoroughly mixed, and 10 μ L of the second cultivation system was taken, added to a third sterile 96-well plate, and then incubated at 30°C for 24 h to obtain a third cultivation system. 1 μ L of the third cultivation system in the third sterile 96-well plate was taken, spotted on YPD plates with 1.0 mg/mL and 4 mg/mL G418, and further incubated at 30°C for 96 h to 120 h. If *Pichia pastoris* transformants could grow on a plate with a high G418 concentration (4 mg/mL), it indicated that the transformants carried a plurality of copies of a target gene, that is, a plurality of recombinant fragments had entered *Pichia pastoris* and had been integrated into a chromosome of *Pichia pastoris* through homologous recombination. After the screening in this step, a recombinant engineered *Pichia pastoris* strain with a high copy number and a high expression level was obtained.

The two engineered strain samples respectively carrying pPIC9K-COL1A1M1 and pPIC9K-COL2A1M6 constructed were deposited in the China General Microbiological Culture Collection Center (CGMCC).

The engineered strain carrying the recombinant expression vector pPIC9K-COL1A1M1 could express the recombinant collagen α 1 (I) M1, and was deposited in the China General Microbiological Culture Collection Center (CGMCC) located at NO. 1, West Beichen Road, Chaoyang District, Beijing, China on March 11, 2021, with an accession number of CGMCC NO.

21891 and a taxonomic name of *Pichia pastoris*.

The engineered strain carrying the recombinant expression vector pPIC9K-COL2A1M6 could express the recombinant collagen $\alpha 1$ (II) M6, and was deposited in the China General Microbiological Culture Collection Center (CGMCC) located at NO. 1, West Beichen Road, Chaoyang District, Beijing, China on March 11, 2021, with an accession number of CGMCC NO. 21892 and a taxonomic name of *Pichia pastoris*.

Example 3 Inducible expression and identification of recombinant collagen

The recombinant engineered strains expressing $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 obtained in Example 2 were taken, and an engineered *Pichia pastoris* strain expressing a full-length type I collagen $\alpha 1$ chain and an engineered *Pichia pastoris* strain expressing a full-length type II collagen $\alpha 1$ chain in the known patents were taken as controls. The two control engineered strains were the previous research results of the team of the inventors, and full-length collagen $\alpha 1$ chains expressed by the two control engineered strains also had a Strep-Tag II at an amino terminus and a $6 \times$ His Tag at a carboxyl terminus. The two control engineered strains were from the application No. 201911135958.0 (title: Yeast Recombinant Human Type I Collagen $\alpha 1$ Chain and Synthesis Method and Use thereof, an engineered *Pichia pastoris* strain expressing a full-length $\alpha 1$ (I) chain in this patent was deposited in the China General Microbiological Culture Collection Center (CGMCC), with an accession number of CGMCC NO. 17150) and the application No. 201911088025.0 (title: Method for Producing Recombinant Human Type II Collagen Single-Chain with *Pichia pastoris*, an engineered *Pichia pastoris* strain expressing a full-length $\alpha 1$ (II) chain in this patent was deposited in the China General Microbiological Culture Collection Center (CGMCC), with an accession number of CGMCC NO. 17149). The four engineered strains each were placed in a 100 mL erlenmeyer flask filled with 10 mL of a BMGY medium, and cultivated at 28°C to 30°C and 220 rpm until OD₆₀₀ was 2 to 6 (16 h to 18 h) to obtain a cultivation system. The cultivation system was centrifuged at 1,500 g to 3,000 g for 5 min at room temperature to obtain a precipitate, the precipitate was resuspended in a BMMY medium to obtain a suspension with OD₆₀₀ of about 2, and then the suspension was cultivated on a shaker at 28°C to 30°C and 220 rpm for 3 d, during which 100% methanol was added to the medium every 24 h to make a final concentration of methanol in the medium was 1.0%. After the induction with methanol was performed for 16 h or more, 1 mL of a strain solution sample was collected, placed in a 1.5 mL EP tube, and centrifuged at 12,000 g and 4°C for 5 min to obtain an expression supernatant, and the expression supernatant was collected and stored at -80°C for later use.

A $5 \times$ loading buffer (250 mM Tris-HCl, pH 6.8, 10% SDS, 0.5% bromophenol blue, 50% glycerin, and 5% β -mercaptoethanol) was added to the collected expression supernatant, and a resulting mixed solution was heated in a metal bath at 100°C for 10 min and then tested by

SDS-PAGE. Because the expressed target protein included a Srtep-Tag II at an amino terminus and a 6 × His Tag at a carboxyl terminus, WB could be performed with an anti-Srtep-Tag II antibody and an anti-6×His Tag antibody (purchased from Nanjing Genscript Biotechnology Co., Ltd.) (specific operations could refer to instructions).

SDS-PAGE results of expression supernatants are shown in FIG. 5. α1 (I) M1, α1 (II) M6, α1 (I), and α1 (II) could be efficiently produced by secretory expression in an extracellular expression supernatant after 24 h of inducible expression. α1 (I) and α1 (II) each have a clear main degradation band (< 116 kDa) below an expected target band (> 116 kDa), while α1 (I) M1 and α1 (II) M6 of the present disclosure each merely have an expected target band (> 116 kDa).

The measurement was performed with the Image Lab software (Bio-Rad Gel Doc XR + Imager), and measurement results were as follows.

(1) An apparent molecular weight (116.3 kDa) of a target band of α1 (I) M1 was substantially consistent with an apparent molecular weight (116.4 kDa) of a target band of α1 (I), and an apparent molecular weight (118.2 kDa) of a target band of α1 (II) M6 was substantially consistent with an apparent molecular weight (118.1 kDa) of a target band of α1 (II). Apparent molecular weights of the target band of α1 (I) M1 and the target band of α1 (II) M6 were significantly larger than an apparent molecular weight of the main degradation band (104.5 kDa) of α1 (I) and an apparent molecular weight of the main degradation band (106.9 kDa) of α1 (II).

(2) A ratio of a target band to a main degradation band in an electrophoresis result of α1 (I) was 51.5% : 48.3%, and a ratio of a target band to a main degradation band in an electrophoresis result of α1 (II) was 52.1% : 47.8%. The two ratios were substantially the same.

It can be seen from enhanced chemiluminescence (ECL) color development results in FIG. 6 (a fully automatic chemiluminescence image analysis system Tanon 5200 integrates protein molecular weight markers into images) that the Srtep-Tag II tag at the amino terminus and the 6 × His Tag at the carboxyl terminus both could be detected in the recombinant collagen α1 (I) M1 and α1 (II) M6, and target bands have the same apparent molecular weights as in SDS-PAGE, indicating that full-length sequences of the recombinant collagen α1 (I) M1 and α1 (II) M6 are successfully and efficiently produced by secretory expression and the expression of target bands is as expected. Although sequences of target bands of α1 (I) and α1 (II) collagens are full-length and complete, main degradation bands of α1 (I) and α1 (II) lack the amino-terminal sequence, and only the carboxyl-terminal 6 × His Tag can be detected.

Target bands of α1 (I) M1 and α1 (II) M6 on an SDS-PAGE pattern, target bands of α1 (I) and α1 (II) on an SDS-PAGE pattern, and main degradation bands were cut off, subjected to enzymolysis with trypsin, and then tested by Nano-HPLC-MS/MS (which was entrusted to Suzhou ProtTech Inc.). Detected peptides were subjected to sequence alignment (Uniprot database), and data alignment results and alignment coverage maps of identified peptides with

native sequences (parts with gray background, indicating parts of a peptide identified by mass spectrometry in a band that were identical to a native sequence) are shown in FIG. 7 and FIG. 8. It could be found that:

(1) Peptides detected after enzymolysis of target bands of $\alpha 1$ (I) M1 and $\alpha 1$ (I) and a main degradation band of $\alpha 1$ (I) all were sequences on a type I collagen $\alpha 1$ chain.

(2) Peptides detected after enzymolysis of target bands of $\alpha 1$ (II) M6 and $\alpha 1$ (II) and a main degradation band of $\alpha 1$ (II) all were sequences on a type II collagen $\alpha 1$ chain.

The above results showed that $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 were expressed as successfully as $\alpha 1$ (I) and $\alpha 1$ (II), and were recombinant collagens of a human type I collagen $\alpha 1$ chain and a human type II collagen $\alpha 1$ chain, respectively, but $\alpha 1$ (I) and $\alpha 1$ (II) were degraded during expression, and main degradation bands of $\alpha 1$ (I) and $\alpha 1$ (II) also were collagens of respective types.

Example 4 High-density fermentation and purification

(1) High-density fermentation with genetically engineered strains

The recombinant collagen $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 were produced by expression on a large scale to obtain fermentation broths containing the recombinant collagen $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6, respectively.

A seed medium YPG (yeast powder: 10 g/L, yeast peptone: 20 g/L, and anhydrous glycerin: 10 g/L); a fermentation medium ($\text{NH}_4\text{H}_2\text{PO}_4$: 190.4 g/L, KH_2PO_4 : 10.06 g/L, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$: 1.18 g/L, K_2SO_4 : 18.2 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 14.9 g/L, and glycerin: 40 g/L); a feed medium (including 50% W/V glycerin and 12 mL of PTM1 trace salts per liter); and an induction medium (including 100% methanol and 12 mL of PTM1 trace salts per liter) were adopted. The PTM1 was filtered through a 0.22 μm filter membrane for sterilization and stored at 4°C. The fermentation medium was sterilized at a high temperature and cooled to room temperature, then PTM1 was added to obtain a PTM1-containing fermentation medium, and the pH of the PTM1-containing fermentation medium was adjusted with ammonia water to 5.0.

Batch-cultivation and inducible expression of the constructed engineered strains were performed as follows.

The fed-batch cultivation was performed at 30°C.

The engineered strain was inoculated into a 1 L shake flask with the seed medium YPG, and cultivated at 220 rpm and 30°C for 18 h to 20 h until OD_{600} was 2 to 10. 5 L fermentation tanks (Baoxing Biological Equipment Co., Ltd.) were used to be filled with 2 L of the fermentation medium containing 2% glycerin, and were sterilized separately. Before inoculation, a rotational speed was adjusted to 300 rpm, a ventilation rate was adjusted to 4 L/min, a temperature was adjusted to 30°C, and a pH was adjusted with an alkaline solution prepared from concentrated ammonia water to 4.5. 0.9 mL of PTM1 was added to the fermentation tank, and then 200 mL of

a seed solution prepared was inoculated in the fermentation tank (flame ring inoculation). Then a dissolved oxygen electrode was clicked for hundred calibration, and fermentation was started. When the dissolved oxygen level fell to 30% for the first time, the dissolved oxygen level was kept at 30% by the dissolved oxygen/rotational speed cascade function. After glycerin was exhausted and the dissolved oxygen level rebounded to greater than 70% (OD₆₀₀ about 20), the dissolved oxygen/rotational speed cascade function was canceled, the stirring speed was increased to 650 rpm, and 80 mL of 30% glycerin was supplemented through linkage feeding. After the glycerin supplementation was completed and the dissolved oxygen level rebounded to 70% or more, the pH was set to 4 and the temperature was set to 29°C to allow induced cultivation with a mixed carbon source of methanol and glycerin (methanol : 50% glycerin = 7:3). 5 mL of the mixed carbon source was manually added. After the dissolved oxygen level rebounded to 70% or more, the feeding rate was set to 8 mL/h, then increased to 10 mL/h one hour later, and then further increased to 20 mL/h one hour later. When the dissolved oxygen level was lower than 30%, the feeding was stopped. After the dissolved oxygen level rebounded to 30%, linkage feeding was started. When a protein concentration measured by a UV method did not increase significantly or decreased after 40 h to 60 h of induction, a resulting fermentation broth was discharged. UV protein quantification formula: C (mg/mL) = 0.144 * (A₂₁₅-A₂₂₅), A₂₁₅ < 1.5. An engineered *Pichia pastoris* strain expressing a full-length α1 (I) chain (which was deposited in the China General Microbiological Culture Collection Center (CGMCC), with an accession number of CGMCC NO. 17150) and an engineered *Pichia pastoris* strain expressing a full-length α1 (II) chain (which was deposited in the China General Microbiological Culture Collection Center (CGMCC), with an accession number of CGMCC NO. 17149) each were used for high-density fermentation.

Results were shown in Table 1. After 48 h of induction, there was no significant difference between α1 (I) and α1 (I) M1 and between α1 (II) M6 and α1 (II) in terms of a strain concentration (OD₆₀₀), a strain wet weight, and a UV-quantified protein concentration expressed in a fermentation broth. Fermentation supernatants were collected and tested by SDS-PAGE, and test results are shown in FIG. 9. Under high-density fermentation conditions, main degradation bands of α1 (I) and α1 (II) were extremely obvious, which had no difference from the inducible expression in shake flasks. Main products of α1 (I) M1 and α1 (II) M6 were still target bands, and no main degradation band occurred, indicating that effects of α1 (I) M1 and α1 (II) M6 for eliminating main degradation bands (main degradation products) could still be effectively maintained under the high-density fermentation conditions.

Table 1 Strain concentrations, strain wet weights, and protein expression levels (UV quantification) in fermentation pilot experiments

Type	OD ₆₀₀	Strain wet weight (g/L)	Protein (UV, g/L)
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$\alpha 1(\text{II})$	189.0	260.0	17.8
$\alpha 1(\text{II})\text{M6}$	198.0	265.0	18.7
$\alpha 1(\text{I})$	215.0	310.0	18.1
$\alpha 1(\text{I})\text{M1}$	201.0	301.0	17.3

(2) Collagen purification

Buffer A: 20 mM KH_2PO_4 , pH 4.0; and

buffer B: 20 mM KH_2PO_4 , 0.5 M NaCl, pH 4.0.

A fermentation broth was collected and centrifuged at 2,000 g and 4°C for 30 min to obtain a strain precipitate and a fermentation supernatant. A cation exchange medium (a chromatography packing was UniGel-80sp produced by Suzhou Nanomicro Technology Co., Ltd., the chromatography packing was loaded on a GCC-50-400 chromatography column produced by the Lisure Science, and a GE AKTA Pure Protein Separation Chromatography Purification System was adopted) was equilibrated with the buffer A until an A215 absorbance value and a conductivity value remained unchanged, and then a sample was loaded at a flow rate of 40 $\mu\text{L}/\text{min}$ and a volume of 0.5 L/time. The UV A215 absorbance was detected, and when it increased, the collection of an effluent was started. When the sample loading was completed, the collection of an effluent was stopped, and then the cation exchange medium was equilibrated with the buffer A. When the A215 absorbance decreased, the collection of an effluent was started until the UV absorbance and conductivity dropped to minimum values and no longer changed. An eluate was collected, tested to determine a composition, and then subjected to dialysis (with ultrapure water as a dialysis solution), concentration, and lyophilization to obtain a lyophilized collagen sponge, and the lyophilized collagen sponge was collected, dissolved in ultrapure water, and subjected to SDS-PAGE. Results are shown in FIG. 10. After one-step ion exchange purification of $\alpha 1(\text{I})\text{M1}$ and $\alpha 1(\text{II})\text{M6}$, most of heteroproteins and small degradation bands were removed, and single target proteins with high purities were obtained (according to measurement results of the Image Lab software, a purity of $\alpha 1(\text{I})\text{M1}$ was 90.1% and a purity of $\alpha 1(\text{II})\text{M6}$ was 88.3%). However, after the same purification step of $\alpha 1(\text{I})$ and $\alpha 1(\text{II})$, a main degradation band still appeared and could not be eliminated, and a main degradation product had a similar size and very similar properties to a target product, such that the main degradation product and the target product could hardly be separated through one-step purification. According to the content in the applications No. 201911135958.0 and No. 201911088025.0, in order to obtain single target full-length $\alpha 1(\text{I})$ and $\alpha 1(\text{II})$ chain products, tandem affinity purification needed to be performed with Ni-NTA and Strep-Tactin affinity chromatography media based on the fact that a full-length $\alpha 1(\text{I})$ chain and a full-length $\alpha 1(\text{II})$ chain had a Strep-Tag II at an amino terminus and a $6 \times \text{His Tag}$ at a carboxyl terminus. In this case, a main

degradation product presented as a main degradation band during SDS-PAGE was discarded, which caused a waste of a biosynthetic resource of a strain, increased purification steps, and reduced a yield of a target product.

Example 5 Detection of recombinant collagens

(1) FT-IR spectroscopy

A trace amount of each of the purified and lyophilized $\alpha 1$ (I) M1, $\alpha 1$ (II) M6, $\alpha 1$ (I), and $\alpha 1$ (II) collagen samples was taken for test, mixed with potassium bromide (KBr), ground into a powder, then pressed into a tablet, and scanned in a range of $4,000\text{ cm}^{-1}$ to 400 cm^{-1} at room temperature (Thermo Scientific, Nicolet™ iS™ 10 FT-IR Spectrometer). The method and result analysis could be found in the literature (Jeong, H., J. Venkatesan and S. Kim, Isolation and characterization of collagen from marine fish (*Thunnus obesus*). *Biotechnology and Bioprocess Engineering*, 2013. 18 (6): p. 1185-1191.).

Infrared (IR) spectra of the purified $\alpha 1$ (I) M1, $\alpha 1$ (II) M6, $\alpha 1$ (I), and $\alpha 1$ (II) protein samples are shown in FIG. 11 and FIG. 12. It can be seen that characteristic absorption average wave numbers of $\alpha 1$ (I) M1, $\alpha 1$ (II) M6, $\alpha 1$ (I), and $\alpha 1$ (II) all were in line with structural characteristics of recombinant collagen: an amide A (about $3,299\text{ cm}^{-1}$), an amide B (about $3,081\text{ cm}^{-1}$), an amide I (about $1,650\text{ cm}^{-1}$), an amide II (about $1,530\text{ cm}^{-1}$ to $1,550\text{ cm}^{-1}$), and an amide III (about $1,240\text{ cm}^{-1}$), indicating that amino acid mutations in $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 did not affect the properties of collagen itself (as shown in literatures [1]. Chen Jingtao et al., *Infrared Spectroscopy of Recombinant Collagen and Bovine Type I Collagen*. *Materials Reports*, 2008 (03): p. 119-121; [2]. Doyle, B.B., E.G. Bendit and E.R. Blout, *Infrared spectroscopy of collagen and collagen-like polypeptides*. *Biopolymers*, 1975. 14 (5): p. 937-957; and [3]. Zhou Aimei et al., *Isolation, Purification, and Structural Characterization of Recombinant Human Collagen*. *Food and Fermentation Industries*, 2015 (03): p. 46-52.).

(2) Detection of a cell adhesion activity of recombinant collagens

A method for detecting a cell adhesion activity of recombinant collagen could be found in the literature: Juming Yao, Satoshi Yanagisawa, Tetsuo Asakura. *Design, Expression and Characterization of Collagen-Like Proteins Based on the Cell Adhesive and Crosslinking Sequences Derived from Native Collagens*, *J Biochem*. 136, 643-649 (2004). The detection of a cell adhesion activity was entrusted to the Functional Nanomaterials and Biomedical Testing Laboratory of School of Pharmacy, Changzhou University.

A specific implementation method was as follows: NIH/3T3 cells purchased from the Cell Bank of the Chinese Academy of Sciences (Product No. GNM6, the cultivation and passage methods were performed according to instructions of the cells) were cultivated normally. Lyophilized $\alpha 1$ (I) M1, $\alpha 1$ (II) M6, $\alpha 1$ (I), and $\alpha 1$ (II) recombinant collagen sponges, control human collagen (purchased from Sigma, Product No. C7774), and bovine serum albumin (BSA,

purchased from Sangon Biotech (Shanghai) Co., Ltd.) each were taken and dissolved with ultrapure water or a 1 M HCl solution, then a protein concentration was determined according to a UV protein quantification empirical formula of $C \text{ (mg/mL)} = 0.144 \times (A_{215} - A_{225})$, and resulting solutions each were diluted with phosphate buffered saline (PBS) (pH 7.4) to 0.5 mg/mL. 100 μ L of each of protein solutions and a blank PBS solution was added to a 96-well cell culture plate, and the plate was allowed to stand for 60 min at room temperature. Then NIH/3T3 cells in a well growth state were added at 10^5 cells/well, and cultivated at 37°C and 5% CO₂ for 60 min. Cells in each well were washed with PBS 4 times. An LDH detection kit (Roche, 04744926001) was used to detect the absorbance OD_{492 nm} (specific operations were performed with reference to instructions).

The absorbance OD_{492 nm} can correspondingly characterize a cell adhesion activity of a collagen sample. The higher the absorbance OD_{492 nm}, the more the cells to which the collagen adheres and the higher the cell adhesion activity, such that the collagen is more likely to help cells adhere to a wall or adhere to an extracellular matrix in a short time, which is conducive to building an excellent extracellular environment. As shown in FIG. 13, the recombinant collagen $\alpha 1$ (I) M1, $\alpha 1$ (II) M6, $\alpha 1$ (I), and $\alpha 1$ (II) all have a similar cell adhesion activity to commercially available native human collagen, and all have a significantly higher cell adhesion activity than the control group, and there is no significant difference between $\alpha 1$ (I) M1 and $\alpha 1$ (I) and between $\alpha 1$ (II) M6 and $\alpha 1$ (II) in terms of the cell adhesion activity.

(3) Preparation and detection of recombinant collagen hydrogels

Recombinant collagens $\alpha 1$ (I) M1, $\alpha 1$ (II) M6, $\alpha 1$ (I), and $\alpha 1$ (II) each were taken and dissolved in water for injection at a concentration of 10% to obtain recombinant collagen solutions, a pH of the recombinant collagen solutions was controlled in a range of 4 to 6, and the recombinant collagen solutions were filtered through a 0.22 μ m sterile filter for sterilization; 0.1 g of a sterile 10% (w/w) N-hydroxysuccinimide (NHS) solution was added per 1 g of a dry collagen powder to obtain first mixed solutions, and first mixed solutions each were thoroughly mixed; then 0.13 g of a sterile 50% (w/w) 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) solution was added per 1 g of a dry collagen powder to obtain second mixed solutions; and the second mixed solutions each were allowed to stand at room temperature (20°C to 30°C) to allow a reaction for 2 h to 6 h to obtain hydrogels. The hydrogels each were subjected to dialysis in a sterile PBS solution (NaCl: 8.5 g/L, Na₂HPO₄: 0.5 g/L, and NaH₂PO₄: 0.15 g/L, pH: 7.2) continuously for 120 h, where a ratio of a hydrogel to a PBS dialysis solution was 1:6 (m/m), and a PBS dialysis solution was completely changed every 24 h to remove NHS and EDC residues. Purified hydrogels produced after the dialysis each were filled in a sterile container and placed at room temperature.

The purified hydrogels each were lyophilized to remove moisture, weighed, then placed in a

sterile PBS solution for 24 h until each hydrogel completely absorbed water and swelled, and then taken out. The moisture on a surface of each swollen hydrogel was removed with absorbent paper, and then the swollen hydrogel was weighed. A swelling ratio was calculated with reference to the method in the literature: $Q_{\text{swelling ratio}} = (W_{\text{water-absorbed swollen gel weight}} - W_{\text{dry gel weight}})/W_{\text{dry gel weight}}$. The elasticity modulus (storage modulus, small amplitude frequency scan, 25°C, stress: 0.5%, and 0.1 rad/s to 100.0 rad/s) and a dynamic viscosity (flow peak hold, 25°C, and shear rate: 2.0 s⁻¹) of a hydrogel were measured by a rheometer (Discovery HR-2). A lyophilized hydrogel was transferred to liquid nitrogen, quickly frozen, broken, and subjected to surface scanning by SEM (Hitachi TM3030PLUS). Elasticity modulus, dynamic viscosity, and swelling ratio results were shown in Table 2. There is no significant difference between hydrogels prepared from $\alpha 1$ (I) M1 and $\alpha 1$ (I) and between hydrogels prepared from $\alpha 1$ (II) M6 and $\alpha 1$ (II) under the same conditions in terms of hydrodynamic properties.

Table 2 Detection results of elasticity moduli, dynamic viscosities, and swelling ratios of four hydrogels

Hydrogel type	Elasticity modulus (Pa)	Viscosity (Pa·S)	Swelling ratio (g/g of a dry hydrogel)
$\alpha 1$ (II)	104.63	82.87	14.18
$\alpha 1$ (II)M6	102.02	77.80	14.01
$\alpha 1$ (I)	234.67	190.67	12.53
$\alpha 1$ (I)M1	292.68	170.97	12.22

As shown in FIG. 14 and FIG. 15, hydrogels prepared from $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 are the same as hydrogels prepared from $\alpha 1$ (I) and $\alpha 1$ (II), respectively, and all have a porous network structure with a pore size in a range of 100 μm to 200 μm , excellent permeability, and a spatial structure basis for retaining a large amount of moisture. Thus, the hydrogels prepared from $\alpha 1$ (I) M1 and $\alpha 1$ (II) M6 can provide a space for adhesion, support, growth, and migration of cells and serve as a channel for delivering nutrients and metabolites, and have the potential of being used in the field of biomedical materials.

(4) Cell detection of recombinant collagen hydrogels

Hydrogels stored aseptically each were placed in a 24-well cell culture plate. NIH/3T3 cells cultivated normally purchased from the Cell Bank of the Chinese Academy of Sciences (Product No. GNM6, the cultivation and passage methods were performed according to instructions of the cells) were taken, washed with PBS, and digested with trypsin, then a medium was added to obtain a cell suspension, and the cell suspension was pipetted up and down for thorough mixing and then counted. The cell suspension was inoculated at 10⁵ cells/well into the cell culture plate coated with the hydrogels and cultivated for 24 h to 72 h, and the adhesion and proliferation of cells on the hydrogels were observed.

(1) A 24-well plate was taken, 1 mM Calcein AM (purchased from the Research Institute of

Beyotime Biotechnology) solution was prepared with DMSO and diluted with D-PBS to obtain a 50 μ M calcein working solution. The medium in wells of the cell culture plate was removed, and the wells were washed with PBS several times. Then 1 mL of a serum-containing DMEM medium and 100 μ L of the Calcein AM solution (1/10 of the medium) were added to the cell culture plate, and the cell culture plate was incubated for 30 min to allow the staining of cells. Then the medium was changed, the cells were cultivated for 30 min, and the hydrogel was gently taken out, placed in a new culture well, and photographed under a fluorescence microscope (a maximum excitation wavelength was 494 nm and a maximum emission wavelength was 514 nm).

(2) Another 24-well plate was taken, 200 μ L of an MTT solution (purchased from the Research Institute of Beyotime Biotechnology) was added to the 24-well plate, the NIH/3T3 cells were cultivated for 4 h, and the production of blue-purple crystals in the cells was observed. The medium was discarded, and the hydrogel was washed with PBS, cut longitudinally, placed in a new culture well, and photographed under a microscope.

The experiments in this example were entrusted to the Functional Nanomaterials and Biomedical Testing Laboratory of School of Pharmacy, Changzhou University.

Results are shown in FIG. 16 and FIG. 17. The hydrogels prepared from α 1 (I) M1 and α 1 (II) M6 are the same as the hydrogels prepared from α 1 (I) and α 1 (II), and NIH/3T3 cells have a normal morphology under a brightfield microscope, which is a typical fibroblast morphology. After NIH/3T3 cells growing adherently on a hydrogel are stained with Calcein AM, green fluorescence can be detected (bright parts in an image). After MTT is added to NIH/3T3 cells growing in a hydrogel, blue-purple crystals can be produced (dark parts in an image). The green fluorescence and the blue-purple crystals can only be produced by viable cells. It indicates that NIH/3T3 cells can adhere to, grow, and migrate normally in the hydrogels. The hydrogels prepared from α 1 (I) M1 and α 1 (II) M6 have similar biological functions to the hydrogels prepared from α 1 (I) and α 1 (II) with native sequences, and thus can be used as novel biomedical devices in fields such as wound healing and tissue regeneration.

CLAIMS

1. A recombinant collagen $\alpha 1$ chain, wherein the recombinant collagen $\alpha 1$ chain is $\alpha 1$ (I) M1 or $\alpha 1$ (II) M6; the $\alpha 1$ (I) M1 is obtained through an amino acid mutation in a native full-length amino acid sequence of a human type I collagen $\alpha 1$ chain, and the $\alpha 1$ (II) M6 is obtained through an amino acid mutation in a native full-length amino acid sequence of a human type II collagen $\alpha 1$ chain; and the $\alpha 1$ (I) M1 has a sequence as set forth in SEQ ID NO: 2, and the $\alpha 1$ (II) M6 has a sequence as set forth in SEQ ID NO: 5.

2. A nucleotide encoding the recombinant collagen $\alpha 1$ chain according to claim 1.

3. The nucleotide according to claim 2, wherein a nucleotide sequence encoding the $\alpha 1$ (I) M1 is set forth in SEQ ID NO: 3; and a nucleotide sequence encoding the $\alpha 1$ (II) M6 is set forth in SEQ ID NO: 6.

4. A recombinant expression vector comprising the nucleotide according to claim 2.

5. An engineered strain carrying the recombinant expression vector according to claim 4 or expressing the recombinant collagen $\alpha 1$ chain according to claim 1, wherein the engineered strain is *Pichia pastoris*.

6. The engineered strain according to claim 5, wherein the engineered strain is deposited in the China General Microbiological Culture Collection Center (CGMCC), with an accession number of CGMCC NO. 21891 or CGMCC NO. 21892, wherein the engineered strain with the accession number of CGMCC NO. 21891 expresses the recombinant collagen $\alpha 1$ chain $\alpha 1$ (I) M1, and the engineered strain with the accession number of CGMCC NO. 21892 expresses the recombinant collagen $\alpha 1$ chain $\alpha 1$ (II) M6.

7. A use of the recombinant expression vector according to claim 4 or the engineered strain according to any one of claims 5 and 6 in a preparation of the recombinant collagen $\alpha 1$ chain.

8. A preparation method of the recombinant collagen $\alpha 1$ chain according to claim 1, comprising the following steps:

(1) synthesizing a nucleotide sequence encoding the recombinant collagen $\alpha 1$ chain according to claim 1;

(2) constructing a recombinant expression vector;

(3) constructing engineered strains for an inducible expression, and screening the engineered strains to obtain an engineered strain with a high expression level;

(4) cultivating the engineered strain with the high expression level for a high-density fermentation to obtain a fermentation supernatant; and

(5) purifying the fermentation supernatant, followed by lyophilization, to obtain the recombinant collagen $\alpha 1$ chain.

9. The preparation method according to claim 8, wherein the engineered strain with the high

expression level is *Pichia pastoris*, and is deposited in the China General Microbiological Culture Collection Center (CGMCC), with an accession number of CGMCC NO. 21891 or CGMCC NO. 21892.

10. A composition, comprising the recombinant collagen $\alpha 1$ chain according to claim 1 or a recombinant collagen $\alpha 1$ chain prepared by the preparation method according to any one of claims 8 and 9.

11. An article, comprising the recombinant collagen $\alpha 1$ chain according to claim 1, a recombinant collagen $\alpha 1$ chain prepared by the preparation method according to any one of claims 8 and 9, or the composition according to claim 10, wherein the article is selected from the group consisting of a drug, a medical device, a biological material, a tissue-engineered product, and a cosmetic.

12. The article according to claim 11, wherein the article is a material for providing an adhesion, a support, and a growth and migration space for a cell or a material serving as a channel for delivering a nutrient and a metabolite.

13. The article according to claim 12, wherein the article is a collagen hydrogel.

14. A use of the recombinant collagen $\alpha 1$ chain according to claim 1, the nucleotide according to any one of claims 2 and 3, the recombinant expression vector according to claim 4, the engineered strain according to any one of claims 5 and 6, a recombinant collagen $\alpha 1$ chain prepared by the preparation method according to any one of claims 8 and 9, or the composition according to claim 10 in a manufacture of a product, wherein the product is selected from the group consisting of a drug, a medical device, a biological material, a tissue-engineered product, and a cosmetic.

15. A use of the recombinant collagen $\alpha 1$ chain according to claim 1, the nucleotide according to any one of claims 2 and 3, the recombinant expression vector according to claim 4, the engineered strain according to any one of claims 5 and 6, a recombinant collagen $\alpha 1$ chain prepared by the preparation method according to any one of claims 8 and 9, or the composition according to claim 10 in a manufacture of a product for promoting wound healing or tissue regeneration.

16. The use according to claim 14, wherein the product is a collagen hydrogel.

17. The use according to claim 15, wherein the product is a collagen hydrogel.