·综 述·

胶原蛋白的开发与应用研究进展

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摘 要：胶原蛋白(collagen)是一类哺乳动物细胞外基质中的主要结构蛋白，广泛地存在于皮肤、骨骼、肌肉等组织中，主要参与细胞的增殖、分化、迁移和信号传递等生理生化行为，对组织细胞等起着支撑、修复、保护的作用。由于胶原蛋白具有良好的生物学特性，其在组织工程、临床医学、食品工业、包装材料、化妆品、医学美容、生物材料以及医疗器械等方面都有着广泛的应用。本文综述了胶原蛋白的生物学特性及其在国内外生物工程研究开发中的研究进展，并对胶原蛋白未来开发的前景进行了展望。

关键词：胶原蛋白；生物工程；开发与应用；研究进展

Research, development and application of collagen: a review

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Abstract: Collagen, which widely exists in skin, bone, muscle and other tissues, is a major

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structural protein in mammalian extracellular matrix. It participates in cell proliferation, differentiation, migration and signal transmission, plays an important role in tissue support and repair and exerts a protective effect. Collagen is widely used in tissue engineering, clinical medicine, food industry, packaging materials, cosmetics and medical beauty due to its good biological characteristics. This paper reviews the biological characteristics of collagen and its application in bioengineering research and development in recent years. Finally, we prospect the future application of collagen as a biomimetic material.
Keywords: collagen; biotechnology; development and application; research progress

胶原蛋白是哺乳动物组织中含量最丰富的蛋白质，占机体总蛋白含量的 30%。同时也是细胞外基质的重要组成成分，维持皮肤、软骨、肌腱、韧带和内脏等组织器官的结构和功能。其具有生物可降解性、低免疫原性及促细胞增殖分化的特性，是一类理想的生物医用和美容护肤材料[1]。传统的胶原蛋白主要通过热水浸提法、酸碱水解法和酶解法等从陆生动物结缔组织和水产加工副产物中提取。但是动物源胶原蛋白的分离纯化工艺复杂且单体分离较难，还可能携带病毒，存在安全隐患，在一定程度上限制了动物源胶原蛋白的应用和开发[2]。而利用基因工程技术生产胶原蛋白具有提取法不具备的优点，如产品纯度、稳定性及安全性高，是目前的研究热点。本文综述了近年来胶原蛋白在国内外研究中的进展，包括胶原蛋白的结构、分类、生产方法及应用等，重点阐述了胶原蛋白在食品、医药、生物材料及美容护肤领域的应用。

1 胶原蛋白概述

1.1 胶原蛋白的结构

胶原蛋白是由多个原胶原组装形成的纤维状蛋白质。原胶原是胶原蛋白的基本单位，由 3 条多肽链组成。原胶原肽链的一级结构具有 $(Gly\text{-}X\text{-}Y)_n$ 重复单位，其中 X 和 Y 常为脯氨酸和羟脯氨酸，少数为赖氨酸和羟赖氨酸。3 条原胶原肽链可通过链间氢键彼此盘绕形成稳定的三螺旋结构[3] (图 1)。在三螺旋构象稳定过程中羟脯氨酸羟基能形成强氢键，可提高胶原蛋白强度；脯氨酸和羟脯氨酸能使胶原三螺旋结构发生急剧扭曲，提高胶原蛋白的稳定性。

1.2 胶原蛋白的分类、分布及功能

目前，已从 40 余种脊椎动物中鉴定出胶原蛋白基因，编码约 29 种类型的胶原蛋白(表 1)。基于一级结构、三螺旋结构域的长度、分子量、三重螺旋结构中断和末端结构域的大小和形状，胶原蛋白可分为 4 大类：第 1 类纤维型胶原，有 I、II、III、V、XI、XXIV 和 XXVII 型，其中 I、II 和 III 型在脊椎动物体内分布较多，V 和 XI 型分布较少，但对 I、II 和 III 型组装起辅助作用；XXIV 和 XXVII 型在三螺旋结构域延伸较短时中断。III、V 和 XI 型胶原经加工后保留 C 端肽和部分 N 端肽结构域；第 2 类三螺旋纤维型胶原(FACIT)，有 IX、XII、XIV、XVI、XIX 和 XXII 型。FACIT 胶原并不形成胶原，但可与原纤维胶原相互作用，调节胶原纤维的形成和大小，并控制细胞外基质中的胶原合成；第 3 类网状型胶原，有 IV、VII 和 XXVIII 型。其中 IV 型胶原蛋白形成纤维状网状结构，而 VII 型胶原组装成锚定纤维，将表皮连接到真皮；第 4 类间断螺旋型胶原(MACITs)，有 XIII、XXIII 和 XXV 型。其中 XIII、XXIII 和 XXV 型胶原是 II 型跨膜蛋白，由 1 个疏水的跨膜结构

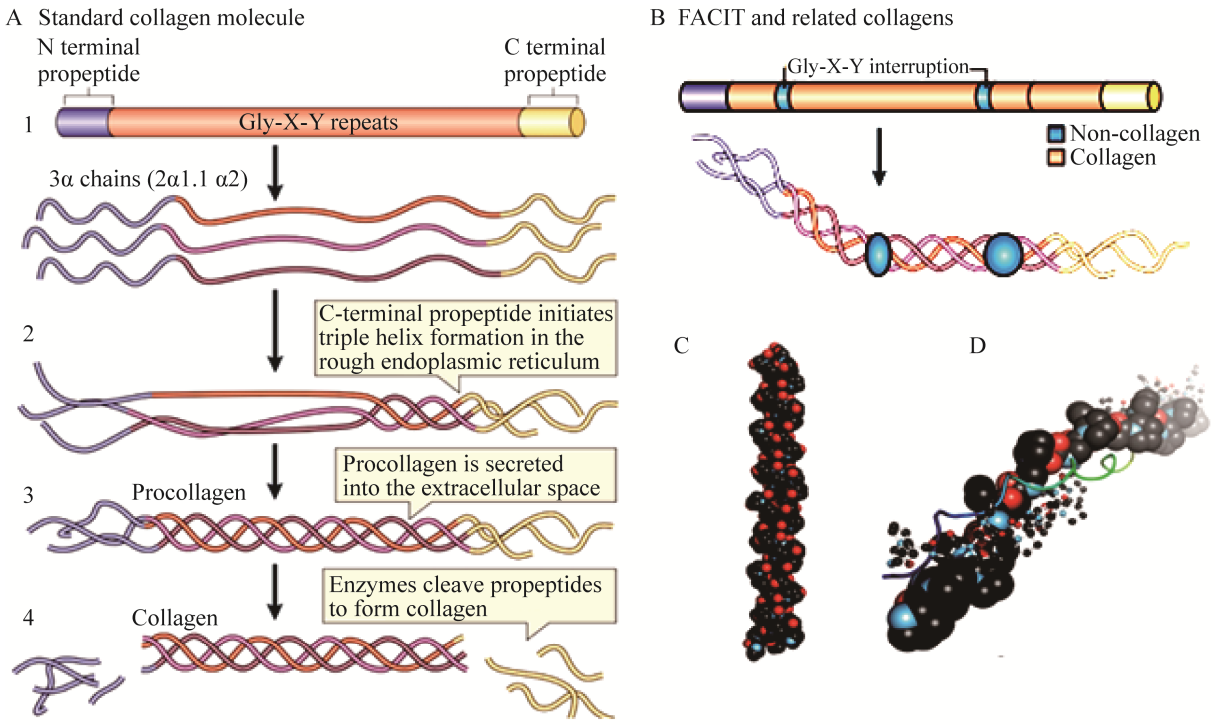
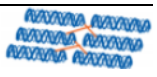


图 1　胶原蛋白的结构[3]

Figure 1　Structure of collagen[3]. A: The standard fiber collagen molecule consists of three α chains. The chain of Gly-*X*-*Y* repeat sequence (step 1) is assembled into triple helix procollagen (remove n-polypeptide, secreted into extracellular space by cells (step 3) and collagen c-procollagen of metalloproteinase (step 4) through the C-terminal domain (step 2). B: Triple helix fibrillary collagen (FACIT), which contains non collagen regions-that is, non triple helix sequences, these clues kink to produce macromolecular structures. C: Collagen triple helix crystal structure, formed by (prohypgly) 4-(prohypala)-(prohypgly) 5. D: Collagen triple helix spatial structure. It is composed of three strands of shaft, ball and bar, and ribbon.

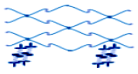
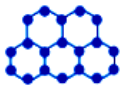
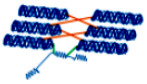
表 1　胶原蛋白的分类、组织分布和结构

Table 1　Classification, tissue distribution and structure of collagen

Structure type	Collagen type	Gene symbol	Chain composition	Supramolecular structure	Tissue distribution	Structure chart
Fibrillar collagens	I	*COL1A1*	[a1(I)]2a2(I)	67 nm banded fibrils	Bone, tendon, dermis, cornea	
		COL1A2	[a1(I)]3			
	II	*COL2A1*	[a1(II)]3		Cartilage, vitreous body of eye	
	III	*COL3A1*	[a1(III)]3		Dermis, aorta, uterus, intestine	
	V	*COL5A1*	[a1(V)]2a2(V)	9 nm banded fibrils	Placenta, bone, dermis, cornea	
		COL5A2	[a1(V)a2(V)a3(V)]			
		COL5A3	[a1(V)]3			
	XI	*COL11A1*	[a1(XI)a2(XI)	Fine fibrils (similar totype V	Cartilage, intervertebral disk	
		COL11A2	a3(XI)]	collagen fibrils)		
	XXIV	*COL24A1*	[a1(XXIV)]3	Presumed to form	Cornea, bone	Unknown
	XXVII	*COL27A1*	[a1(XXVII)]3	homotrimeric fibrils	Cartilage, eye, ear, lung, colon	

(待续)

(续表 1)

Structure type	Collagen type	Gene symbol	Chain composition	Supramolecular structure	Tissue distribution	Structure chart
Basement membrane collagens	IV	*COL4A1* *COL4A2* *COL4A3* *COL4A4* *COL4A5*	[a1(IV)]2a2(IV) [a1(IV)]2a3(IV) [a1(IV)]2a4(IV) [a1(IV)]2a5(IV) [a1(IV)]2a6(IV)	Nonfibrillar meshwork	Basement membranes	
	VII	*COL7A1*	[a1(VII)]3	Anchoring fibrils	Skin, amniotic membrane, mucosal epithelium	
Short-chain collagens	VI	*COL6A1* *COL6A2* *COL6A3*	[a1(VI)a2(VI) a3(VI)]	5–10 nm beaded microfibrils	Uterus, dermis, cornea, cartilage	
	VIII	*COL8A1* *COL8A2*	[a1(VIII)] 2a2(VIII)	Nonfibrillar hexagonal lattice	Endothelial cells, descemet's membrane	
	X	*COL10A1*	[a1(X)]3		Calcifying cartilage	
FACIT collagens	IX	*COL9A1* *COL9A2* *COL9A3*	[a1(IX)a2(IX) a3(IX)]	Associates with type II collagen fibrils	Cartilage, vitreous body of eye	
	XII	*COL12A1*	[a1(XII)]3	Associates with type I collagen fibrils	Dermis, tendon, cartilage	
	XIV	*COL14A1*	[a1(XIV)]3	Associates with type I and II collagen fibrils		
	XVI	*COL16A1*	a1(XVI)	Associates with fibrillin-1 rich microfibrils and D banded type II collagen fibrils	Heart, kidney, smooth muscle	
	XIX	*COL19A1*	a1(XIX) [a1(XIX)]2 [a1(XIX)]3 [a1(XIX)]4 [a1(XIX)]5 [a1(XIX)]6	N-terminal-interacting oligomers	Basement membrane of differentiating muscle cells	
	XX	*COL20A1*	a1(XX)	Presumed to bind to collagen fibrils	Corneal epithelium	
	XXI	*COL21A1*	a1(XXI)	Unknown	Placenta, vasculature of heart, stomach, kidney, skeletal muscle	
	XXII	*COL22A1*	a1(XXII)	Presumed to bind to basement membrane components	Myotendinous junction, articular cartilage-synovial fluid junction, hair follicles	
MACITs collagens	XIII	*COL13A1*	a1(XIII)	Support skin	Endothelial cells, epidermis	
	XVII	*COL17A1*	a1(XVII)	Binding to basal cells and matrix	Epidermal and endothelial junction of skin	
	XXIII	*COL23A1*	a1(XXIII)	Unknown	Tumor (prostate)	
	XXV	*COL25A1*	a1(XXV)	Unknown	Role of R amyloid plaques in Alzheimer disease	
Unclassified collagens	XXVI	*COL26A1*	a1(XXVI)	Unknown	Testis, ovary	Unknown
	XVIII	*COL18A1*	a1(XVIII)	Highly vascularized tissue expression	Lung, liver and kidney	Unknown
	XV	*COL15A1*	a1(XV)	Participation expression	Fibroblasts, endometrium	Unknown
	XXVIII	*COL28A1*	a1(XXVIII)	Unknown	Basement membrane, peripheral nervous system	Unknown
	XXIX	*COL29A1*	a1(XXIX)	Unknown	Epidermis, lung, small intestine	Unknown

域、1 个短的 N 端胞质域和 3 种胶原蛋白组成的胞外域组成。

1.3 胶原蛋白的生物合成

胶原蛋白的生物合成可分为细胞内和细胞外 2 个阶段，如图 2 所示。

1.3.1 细胞内合成阶段

在组织细胞的细胞核(nucleus)中，胶原分子的各个肽链的遗传信息由信使 RNA (mRNA)将编码蛋白所需的信息转录到核糖体(ribosome)，在核糖体上合成 1 000 多个氨基酸残基的肽链，肽链转入内质网(endoplasmic reticulum, ER)中进行羟基化和糖基化修饰。(1) 羟基化修饰：在内质网中，肽链由脯氨酸、赖氨酸残基经脯氨酸羟化酶(prolyl hydroxylase)和赖氨酸羟化酶(lysyl hydroxylase)催化生成，羟化作用对三股螺旋的坚固性有重要作用，羟化不足的肽链在体温下不能形成坚固的三股螺旋，因而不能分泌至细胞外。(2) 糖基化修饰：在内质网中，肽链由半乳糖基转移酶及葡萄糖基转移酶催化将糖基连于 5-羟赖氨酸残基上形成，该修饰有利于纤维的定向排列。经羟基化和糖基化修饰后的溶胶原蛋白，可形成三股螺旋前胶原而分泌至细胞外。

1.3.2 细胞外胶原纤维成熟阶段

三股螺旋前胶原分泌到细胞外的溶胶原经内切酶作用后，水解 N-末端和 C-末端的附加肽链，形成原胶原蛋白，原胶原分子可在中性 pH 条件下，借分子间各部分不同电荷的相互吸引而自动聚合成胶原纤维，该聚合不稳定，需由赖氨酸氧化酶(lysyl oxidase)催化，将赖氨酸转变为醛赖氨酸(allysine) ε-醛赖氨酸后，首先与 α-肽链上 ε 赖氨酸醛缩合生成 ε-醛赖氨酸醛酸(allysine aldol)，然后与组氨酸反应生成醇醛组氨酸(aldol histidine)，后者再与 5-羟赖氨酸进行醛胺缩合形成席夫碱结构可使 4 条 α-肽链间共

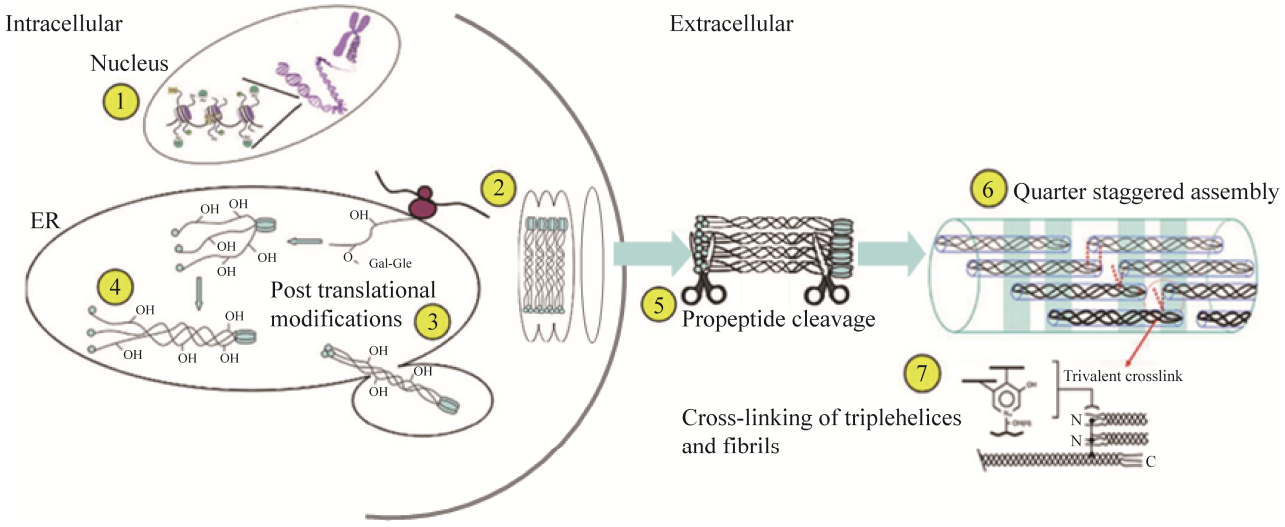


图 2 胶原蛋白在细胞内外的生物合成过程[4]

Figure 2 The biosynthesis process of collagen inside and outside human tissue cells[4]. ①: Genetic informations of collagen polypeptide chains are transcribed in the nucleus; ②: Collagen polypeptide chain transferred into endoplasmic reticulum; ③: Hydroxylation and glycosylation modification of collagen polypeptide chain; ④: Collagen polypeptide chain forms triple helix procollagen; ⑤: Procollagen of triple helix is formed into procollagen by endonuclease outside cells; ⑥: Procollagen automatically polymerizes into collagen fibers; ⑦: Collagen fibers form insoluble collagen fibers through covalent crosslinking.

价交联,使得胶原微纤维的张力加强,韧性增大,溶解度降低,最终形成不溶性的胶原纤维[5]。

2 胶原蛋白的生产

天然胶原蛋白原料主要来源于陆生动物和水生生物加工后的副产物。陆生动物源胶原蛋白主要指从猪、牛的皮和骨中提取的胶原蛋白。然而,由于疯牛病、口蹄疫等牲畜疾病的存在,导致此类胶原存在人兽共患病的风险。近年来的研究发现鱼皮、鱼骨和鱼鳞中也富含胶原蛋白。从水生动物组织中提取胶原蛋白不仅能够充分利用我国的水产资源,而且可以减少水产加工副产物对环境的污染[5]。因此,水生生物源胶原蛋白越来越受到人们的关注。此外,随着基因工程、蛋白质工程等分子生物学研究的快速发展和成熟,重组胶原蛋白研究也快速推进。与传统的提取法相比,重组胶原蛋白产品纯度、稳定性及安全性更高。然而,不同来源的胶原蛋白,其制备工艺和结构性质存在差异。

2.1 传统提取法

胶原传统的提取方法有热水浸提法、酸碱水解法和酶解法。热水浸提法以纯水作为溶剂,通常在高温(>150 ℃)和高压(25 000 kPa)下进行。与化学水解法和酶水解法相比,热水浸提法环保,且操作工艺简单、经济、无毒且安全,是一种经济高效且可持续的提取技术。酸水解法通常以酸为介质(多为醋酸、盐酸、乳酸或柠檬酸),从动物组织(猪皮、牛皮、驴皮、鱼等)中提取胶原蛋白。而碱水解法常以 $Ca(OH)_2$ 和 NaOH 为溶剂,其蛋白提取得率低于酸水解法。然而,酸碱水解法都容易导致胶原活性丧失,污染环境。酶解法则是利用胃蛋白酶、胰蛋白酶和木瓜蛋白酶等将胶原分子端肽间的共价键切除,促进胶原蛋白的溶出。其反应条件相对温和,不产生有毒物质,对胶原蛋白活性的破坏较小,被广泛应用于食品和制药行业。

2.2 化学合成法

提取法获得的胶原蛋白在某些生物材料应用中改性较差,易致病和产生免疫排异反应。近年来,合成化学的发展为这些问题提供解决方案。Gómez-Guillén 等[6]利用三螺旋倾向自组装技术模拟天然胶原蛋白结构和热行为产生原纤维,通过调节氨基酸组成、温度和溶剂来控制胶原蛋白的稳定性和自组装长度。Falk 等[7]将 Fe^{2+}添加到三螺旋胶原相关肽(collagen-related peptide, CRP)的溶液中触发自组装成形态多样的原纤维。化学合成法产生的三螺旋胶原蛋白,虽然解决了免疫排异和病毒隐患的问题,但是合成的技术比较复杂,成本较高,且无生物活性。因此,仅限于实验室研究。

2.3 基因工程方法

基因工程技术生产胶原蛋白又称为重组胶原蛋白,是指基于人胶原蛋白的特征和主要功能域重新优化设计基因序列,然后通过选用各种宿主细胞,如转基因鼠、昆虫、转基因蚕、转基因烟草、大肠杆菌、酵母等生产重组人源胶原蛋白(表 2)。该技术生产的胶原蛋白具有安全性高、批次稳定、组分单一、活性高、无免疫排异等优点。不仅规避了传统提取方法存在的风险,还提高了胶原蛋白的亲水性能(表 3)。常用的宿主细胞包括大肠杆菌、酵母和转基因动植物,不同宿主表达系统有不同的特点。其中,动植物细胞的发酵培养难度大、纯化成本高,且产量低,不适宜于大规模生产,仅限于实验室研究。微生物培养及发酵成本较低,遗传背景清晰,操作性强,适宜工业化生产。

西北大学范代娣课题组成功利用大肠杆菌基因工程菌 BL21(DE)发酵生产并分离纯化了类人胶原蛋白,改进了动物体胶原蛋白的水溶

表 2 生产重组胶原蛋白的表达体系

Table 2 Expression system for producing recombinant collagen

Expression system	Type	Expressed genes; expression vector; host; expression quantity/proportion of total protein	Application	Source
E. coli	II	*COLFc*; pET30a; BL21(DE3)/pLysS & BL21(DE3) Rosetta; 13 mg/L	Cartilaginous	[8]
	VI	*CP6*; pET30a; BL21(DE3); 31.52 mg/L	Medicine, beauty, cosmetics, etc	[9]
	VI	*CW-CP6*; pET-32a; BL21(DE3)/pLysS, Rosetta(DE3); 20 mg/mL	Photoaging resistance	[10]
	VI	*Co16A2*; pET22b; BL21(DE3); 30.5%	Artificial blood vessel & surgical suture	[11]
	I	*rhCol*; pET28a; Rosetta(DE3); 520 μg/mL	Antioxidation & promote cell proliferation	[12]
	III	*rhCol*; pET30a(+)-1880/pACYCDuet; BL21(DE3); 3 g/L	Skin care & biomedicine	[13]
	II	*hCII250–270*; pUC19; BL21(DE3); 30%	Rheumatoid arthritis	[14]
	III	*Kit*; pET-28a(+); BL21(DE3); 3.02 g/L	chemical industry and biomedicine	[15]
	II	BL21(DE3); 10.8 g/L	Promote bone healing	[16]
	IV	α_1 chain NC1; pQE-31; M15(pREP4); 32%	Tumor angiogenesis inhibition therapy	[17]
	IV	α_1 chain NC1; pPROEXHTb; BL21(DE3); 32%	Inhibiting tumor growth and metastasis	[18]
	VI	*Col6A2*; pET32a; BL21(DE3)/pLysS/Rosetta(DE3); 34.2%	Antioxidation	[19]
	I	*X21ex*; pET28a; BL21(DE3); 35%	Cosmetology	[20]
	I	*hCII250–270*; pET3C; BL21(DE3); 48%	Wound healing and repair	[21]
P. pastoris	III	α_1 gene sequence; pPIC9KG6; GS115; 60.1 mg/L	Maintain physiological, repair cells, tissues and organs	[22]
	VI	*Col6A2-005*; pPIC9K; GS115; 39.77 mg/L	Congenital muscular dystrophy	[23]
	III	*pC7*/*P4H*; pPICZαA; X33; 0.2 g/L	Coexpression	[24]
	III	*Gel*; pPIC9KG6; GS115; 98.06 μg/ml	Unknown	[25]
	III	α_1(I) chain; pPICZα; X33; 1.47 g/L	Vaccine stabilizer	[26]
S. cerevisiae	III	Unknown	Cosmetics	[27]
Other	III	α_1 gene sequence; pMG36e; *S. thermophilus*; 1.97%	Whitening, promoting bone healing, food health care	[28]
	I	*Col R75E*; pPICZαA; X-33; 4.977 U/mg	Medical and food	[29]
	I	α_1(I); mammary gland cells of transgenic mice; 8 mg/mL	Milk degradation	[30]
	III	*BmP4Hα*; PSG cell; 40–60 mg/L	Cosmetics	[31]
	I	α_1(I); pPICZB; 8 mg/mL	Substitute animal gelatin	[32]
	II	3T3 cell	Unknown	[33]
	III	Virus system	Unknown	[34]

表 3　重组胶原蛋白与天然胶原蛋白的特性对比[35]

Table 3　Comparison of characteristics between recombinant collagen and natural collagen[35]

Characteristics	Recombinant collagen	Natural collagen
Affinity with human body	Homogeneous collagen, high human affinity	Allogeneic collagen, weak human affinity
Allogeneic collagen, weak human affinity	Genetic engineering and controllable quality	Poor chemical extraction and controllability
Safety	Virus free yeast fermentation	Easy to carry animal virus
Susceptibility to allergy	Homogeneous protein, not susceptible to allergy	Heterologous protein, allergic
Biological activity	It has collagen spatial structure and retains biological activity	No collagen spatial structure, no biological activity
Purity	Single collagen, fixed components, purity up to 95%	Mixed collagen with complex components
Moisture retention	High	Low
Water solubility	Soluble in water	Insoluble in water
pH value	6.7–7.5	3.0–4.5

性、免疫排异性、吸收性等特性，并通过和西安巨子生物合作进行规模化生产[36]。同时该课题组从人胎盘组织中提取 mRNA，反转录为 cDNA，并以其为模板克隆出 I 型胶原 α1 链基因后，与表达载体 pPIC9K 连接构建质粒 pPIC9K-Col1a1，然后质粒 pPIC9K-Col1a1 酶切线性化后转入毕赤酵母菌 SMD1168 中得到转基因毕赤酵母基因工程菌，通过诱导表达出胞外分泌的人 I 型胶原 α1 链蛋白，该蛋白与其他无机材料混合，可作为生物可降解材料，用于组织缺损和修复等[37]。四川大学和山西锦波生物医药股份有限公司共建“川大-锦波功能蛋白联合实验室”开发了 III 型重组人源胶原蛋白的制备工艺，其制备方法主要通过选取人胶原基因 *COL3A1* 的关键片段区域 483–512 aa 进行重复拼接，并在大肠杆菌工程菌中诱导表达。所得的重组人源化 III 型胶原蛋白水溶性强、生物活性高[38]。本课题组则选取 *COL3A1* 基因中富含活性位点的区段 428–1 172 aa 进行克隆，并在毕赤酵母中进行表达。此外，本课题组还完成了类胶原蛋白、I 型胶原蛋白、II 型胶原蛋白等一些重要的胶原蛋白原料的重组表达及产业化应用。

3　胶原蛋白的应用

近年来，胶原蛋白已被广泛用于生物支架材料、化妆品、食品和医疗器械等领域，基于胶原蛋白的产品开发主要集中在骨修复、皮肤创面修复敷料、肌腱修复、药物输送和美容等方面。

3.1　创面愈合与组织修复

胶原蛋白作为细胞外基质的重要组成之一，能诱导上皮细胞增殖、分化和迁移。与生物合成敷料相比，胶原复合敷料与成纤维细胞相互作用形成创面收缩力，减少创面的粘连和收缩强度[15]。Cao 等[39]制备了一种类人胶原蛋白-羧甲基壳聚糖(human-like collagen-carboxymethylated chitosan, HRC-CCS)皮肤支架水凝胶，具有良好的促进创面皮肤组织再生能力。Pan 等[40]合成了具有透气性、细菌阻隔性和止血活性的多功能 PVA/HRC/SA 复合水凝胶，能促进全皮层创面愈合。Lei 等[41]以谷氨酰胺转氨酶作为交联剂形成透明质酸(hyaluronic acid, HA)、羧化壳聚糖和类人胶原蛋白的水凝胶混合敷料，可有效预防细菌感染，促进烧伤创面愈合。胶原蛋白具有良好的生物相容性、可降解性及诱导成骨

分化的作用，被用于骨缺损修复。Hamzah 等[42]使用胶原蛋白包埋聚乳酸制成了一种潜在的仿生聚合物 3D 骨支架，可作为骨组织工程应用材料。James 等[43]将胶原蛋白与羟基磷灰石复合成一种羟基磷灰石矿化胶原复合物，可用作骨传导涂层和支架。在齿科修复治疗中，将胶原蛋白填补到牙周骨质缺损部位，可加速牙周骨质增生和牙龈再生[20]。

此外，鉴于生长因子在创面修复中具有重要的作用，本课题组[21]利用基因工程技术设计和构建了一种包含天然 I 型胶原蛋白的细胞粘附结构域新型的重组类胶原蛋白(recombinant human-source collagen, RHC)，将 RHC 与表皮生长因子(epidermal growth factor, EGF)复合制成冻干敷料，不仅可以保持周围伤口湿润，还可以主动加速伤口愈合过程。由此可见，胶原蛋白广泛参与组织修复和创面愈合的过程，基于胶原蛋白的生物材料在组织修复和创面愈合中扮演着重要的角色。

3.2 化妆品与医学美容

胶原蛋白是皮肤的主要成分，随着年龄的增长，胶原蛋白流失，使得皮肤失去弹性和光泽，甚至会出现色斑等老化现象。因此，胶原蛋白被广泛开发成护肤产品配方。含胶原蛋白的护肤品与皮肤的亲和力强，具有保湿、柔软、抗氧化和紫外线防护等生物活性。在化妆品配方中常用的胶原蛋白为水解胶原蛋白及重组胶原蛋白，与天然胶原蛋白相比，水解胶原及重组胶原的分子量小，在中性条件下具有出色的溶解性、水结合和易渗入真皮的特性[44]。本课题组在前期研究中开发了一种重组类人胶原蛋白(recombinant human-like collagen, rhLEC)[45]及功能结构胶原蛋白(functional-construction collagen, FCC)[46]，在化妆品中添加时具有促进细胞粘附、组织胶原新生、修复受损皮肤的作用。在医学美容方面，胶原蛋白注射剂已广泛用于修复皮肤缺陷及皮下疾病。面部局部注射胶原蛋白可以达到面部轮廓矫正、皱纹、瘢痕修复等效果。目前，国内市场将天然胶原蛋白应用于化妆品及医学美容的企业主要有广州创尔生物、台湾双美、无锡贝迪生物、长春博泰、北京益尔康、上海昊海生科、北京克劳德、哈尔滨沛奇隆等；而重组人源胶原主要厂家为山西锦波生物医药股份有限公司、陕西巨子生物技术有限公司、广东丸美生物技术股份有限公司、广州市暨源生物科技有限公司、广州暨南大学生物医药技术研究开发中心有限公司、北京未名拾光生物技术有限公司、江苏创健医疗科技有限公司、山东华熙生物科技有限公司、江苏江山聚源生物技术有限公司等。其中生产重组人源胶原蛋白的初创企业不断增加，如未名拾光通过基因重组和生物发酵，利用“分子压缩”技术制备出更小的蛋白分子，从而达到普通胶原无法达到的，已建立了植物与人体的天然生物活性分子数据库，并基于此设计并交付了一款来源于胶原蛋白的具有抗衰功能的护肤原料(CN114842916A)[47]，并希望通过合成生物学技术为开发新的医美、护肤产品；江苏创健以开发重组胶原蛋白为使命，致力于重组胶原基生物材料的研发与生产，构建了人 I、II、III、XVII 型胶原蛋白的制备方法，提高了目的产物的产率(CN114106150B[48]、CN114774460A[49]、CN114480471A[50]、CN113185604B[51]、CN110747198B[52]、CN110606896B[53]、CN110964099A[54]和 CN109988243A[55])；江苏江山聚源专注于仿生重组蛋白产业化设计与应用，从事重组人源胶原蛋白及下游产品的研发、生产，构建了重组人源 I、III、IXIII 型胶原蛋白、表达菌株及构建方法(CN111363029B[56]、CN113880940A[57]、CN113880941A[58])。

3.3 食品工业与包装材料

在食品工业中，食品包装材料需要排出氧气和控制水分迁移，在保持食品感官品质的同时，并防止脂肪氧化、变色和微生物侵入。胶原蛋白具有抗氧化活性，其水解物被用于抑制脂质过氧化，降低脂质过氧化对人体造成的影响，可用作薄膜和涂层等包装材料的开发。在保护、维持和延长食品保质期方面发挥了重要作用[59]。如明胶作为一种热变性胶原蛋白，被广泛用作食品添加剂、微胶囊化剂和可生物降解的包装材料。但其机械强度较差、吸湿性高，与高水分食品接触时易膨胀溶解，限制了其在食品包装中的直接应用。而通过化学或物理方法将明胶进行交联或与其他生物聚合物组合，可降低明胶链的流动性，提高其稳定性、耐水性、耐热性、阻隔性和机械性能。

3.4 生物医学材料

胶原蛋白因低免疫原性、可生物降解性、修复特性及止血特性等成为了理想的生物医学材料，广泛应用于医用敷料、骨修复材料(人工骨)、手术缝合及药物载体等领域。胶原蛋白用于生物医学材料，无论组织类型如何，在选择用于组织工程的材料时，应考虑许多关键因素，如生物相容性、生物降解性、机械支撑性等(表 4)，而这些功能的实现将决定支架是否适合用作模拟细胞天然生理环境的生物材料[60]。胶原蛋白作为天然细胞外基质的主要结构成分，具有高生物相容性、生物降解性和延展性；但胶原蛋白通过自身形成的纤维状结构支架表现出较差的机械性能，因此需要对材料进行改性以达到最佳效果。目前，改性策略主要是将胶原蛋白与其他天然聚合物结合，如将胶原蛋白与具有高生物相容性、适度降解和抗菌作用等特性的壳聚糖结合，获得机械性能和生存能力更强的片状结构。与单一胶原蛋白材料相比，将胶原蛋白与天然聚合物共混可开发出一种具有改进的机械性能和生物相容性的新型材料。此外，胶原蛋白还能与不同的聚合物混合用于皮肤、骨骼及黏膜的修复[61] (表 5)。

除二元混合物之外，胶原蛋白与多种不同聚合物制成的混合物也是新趋势。这种材料通常基于细胞、生物活性分子和材料的整合，能充当细胞外基质为组织提供结构支撑、组织再生、允许营养物质和气体的扩散，为细胞增殖

表 4 胶原蛋白作为生物医学材料的设计要求[60]

Table 4 The design requirements of collagen as biomedical material[60]

Critical factor	Selection and design requirements
Biocompatibility	Guarantee normal cell function
	Cause minor immunological reaction *in vitro*
Biodegradability	Allow cells produce their own extracellular matrix (ECM)
	By-products must be non-toxic
Mechanical prop	Match native’s ones
	Permit surgical manipulation
	Consider cell reaction to mechanical forces
Architecture	Maintain integrity
	Allow nutrient and oxygen supply
	Determined by porosity
Manufacturing	Must be sterile
	Clinically as well as commercially feasible

提供所需的微环境[60]。如胶原蛋白、透明质酸和壳聚糖三者复合可用于生产具有独特结构和机械特性的人造混合物(图 3)。本课题组前期根据颅骨创伤性缺陷模型设计了一种 TGF-β3/重组类胶原蛋白 RHC/壳聚糖冻干海绵混合材料[73]，可快速恢复颅骨创伤性缺损，其治疗效果优于单独使用胶原蛋白、壳聚糖或 TGF-β3。因此，不同材料的复配及组合可达到性能互补的效应，而不同组分比例的调配可调控材料的性能，不仅能降低复合材料的生物降解作用，而且还能改变材料的力学性能、药物缓控释性能和抗菌性能等，这是未来胶原蛋白生物材料的重要发展方向之一。

3.5 医疗器械

在医疗器械中，胶原蛋白的主要用途包括创面愈合、皮肤修复、皮炎、湿疹、痔疮、口腔黏膜炎、口腔溃疡、疤痕及过敏性鼻炎等。随着胶原蛋白在医疗器械方面的广泛应用，

表 5 胶原蛋白在生物医学材料方面的应用

Table 5 Application of collagen in biomedical materials

Mixed materials	Add substance	Application type	Function	Source
Collagen/Chitosan	Hydroxyapatite	Electrospinning	Artificial bone	[62]
	Unknown	3D printing stand	Spinal cord injury	[63]
	Silver nanoparticles	Nanoparticles	Wound regeneration	[64]
	Aloe	Microsphere scaffold	Loading vitamins and other active substances	[65]
Collagen/Silk fibroin	Unknown	Tissue engineering	Vascular stent	[66]
	Hydroxyapatite	Biological scaffold	Cell proliferation	[67]
	Hydroxyapatite	Bone tissue engineering	Artificial bone	[68]
Collagen/Hyaluronic acid	Unknown	Tissue regeneration	Tissue repair, cartilage scaffold regeneration	[26]
Collagen/Chitosan/Other	Alginate	Composite dressing	Wound healing	[69]
	Adhesin	Hydrogel	Treatment of type 1 diabetes	[70]
	Gelatin-hyaluronic acid	Biomimetic membrane	Corneal tissue	[71]
	Silk fibroin	3D material support	Promote the adhesion and growth of MG-63 cells	[72]

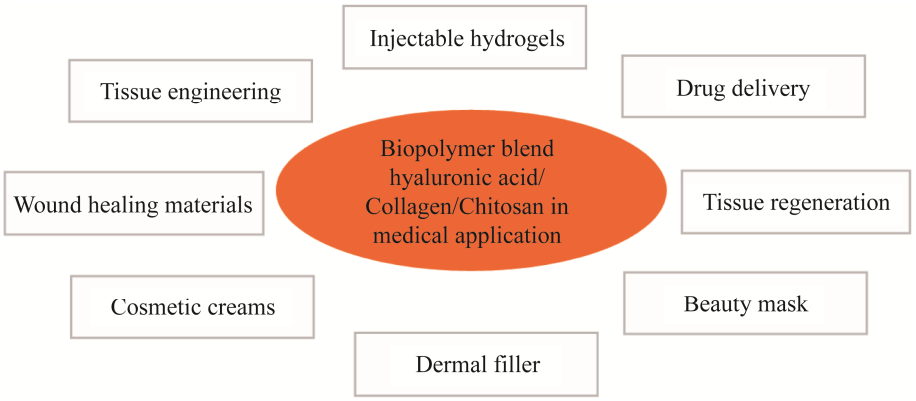


图 3 以透明质酸、胶原蛋白和壳聚糖为基础的生物高聚物混合物的潜在应用[63]

Figure 3 Potential applications of biopolymer mixtures based on hyaluronic acid, collagen and chitosan[63].

2021 年 3 月 15 日，国家药品监督管理局发布《重组胶原蛋白生物材料命名指导原则》[74]对重组人胶原蛋白、重组人源化胶原蛋白和重组类胶原蛋白做出明确定义，并要求企业在医疗器械领域重组胶原蛋白生物材料的命名中采用“核心词 A+核心词 B (表明产品形态)”的形式。2021 年 4 月 15 日，国家药品监督管理局发布《重组胶原蛋白类医疗产品分类界定原则》[75]明确规定重组胶原蛋白类产品的管理类别不低于II类医疗器械。胶原蛋白作为无源植入物，及产品可部分或全部被人吸收或用于体内的止血海绵或医用敷料时按III类医疗器械管理。这标志着对于市场层面重组胶原蛋白的创新和应用，政策层面已显示出更为积极的响应和规范举措，表明相关部门规范行业发展的决心，另一方面也体现了政策对创新产业发展的敏感度和支持力度。目前，国内基于胶原蛋白开发的医疗器械按用途、适应症、产品名称、注册类别和生产厂家归类见表 6。

4 总结与展望

胶原蛋白来源丰富，可以从不同的物种(如牛、猪、家禽和海洋生物)中提取。其中，牛胶原蛋白成本低且提取方便，因此使用最多。虽然海洋胶原蛋白具有较高的吸收率和生物利用度，但由于提取和制造成本高昂，技术尚不完善，无法实现工业化生产，其使用相对有限。此外，利用重组技术生产胶原蛋白也取得了长足的进步，但是目前制备的重组胶原蛋白的产量、羟脯氨酸羟化率、三螺旋结构特性及纯度仍有待提升和改进。随着第三次生物技术革命的合成生物学技术的发展，以“基因调控，工程设计”为核心，从胶原蛋白分子的定向设计、细胞工厂构建与适配调控等方面出发，通过设计、

表 6 胶原蛋白在医疗器械方面的市场应用

Table 6 Market and application of collagen products in medical devices

Company	Product name	Category	Application
Jiyuan Biology	Recombinant type III humanized collagen dressing	II	Wound healing and skin repair
Jiyuan Biology	Recombinant type III humanized collagen liquid dressing	II	
Jiyuan Biology	Recombinant type III humanized collagen compound liquid dressing	II	
Dongwan Biology	Collagen dressing	III	
Zhiyuan Pharmaceutical	Collagen dressing	II	
Chuang’er Biology	Collagen sponge	III	
Kefu Medical	Collagen repair patch	II	
Qianbaina Biology	Human like collagen repair fluid	II	
Qianbaina Biology	Human like collagen repair patch	II	
Qianbaina Biology	Human like collagen repair dressing	II	
Chuangming Medical	Yeast recombinant collagen wound gel dressing	II	
Chuangming Medical	Yeast recombinant collagen gel dressing	II	
Jinbo Biomedicine	Medical type III collagen solution	II	
Yunmeida Biology	Medical recombinant type III humanized collagen repair patch	II	
Chuangming Medical	Yeast recombinant collagen dressing	II	
Chuangming Medical	Yeast recombinant collagen dressing	II	

(待续)

(续表 6)

Company	Product name	Category	Application
Juzi Biology	Human like collagen bioremediation dressing	II	
Juzi Biology	Human like collagen dressing	II	
Meiyan Regenerative Medicine	Medical recombinant type III humanized collagen repair patch	II	
Pu Liyan Biology	Recombinant collagen bioremediation dressing	II	
Elome Biology	Medical recombinant type III humanized collagen solution	II	
Elome Biology	Medical recombinant type III humanized collagen repair patch	II	
Miaote Pharmaceutical	Medical type III collagen dressing	II	
Novo Pharmaceutical	Medical recombinant type III human collagen repair patch	II	
Meiyan Regenerative Medicine	Medical recombinant type III humanized collagen solution	II	
Heineken Heineken Healthcare	Medical recombinant collagen repair patch	II	
Juzi Biology	Human like collagen repair dressing	II	
Juzi Biology	Human like collagen dressing	II	
Chuangjian Medical	Yeast recombinant collagen wound gel dressing	II	
Chuangjian Medical	Yeast recombinant collagen dressing	II	
Weide Medical	Medical collagen application	II	
Beizhuya Pharmaceutical	Collagen dressing	II	Dermatitis and eczema
Chuangjian Medical	Yeast recombinant collagen gel	II	
Chuangming Medical	Yeast recombinant collagen gel dressing	II	
Chuangming Medical	Yeast recombinant collagen liquid dressing	II	
Chuangming Medical	Yeast recombinant collagen dressing	II	
Chuangming Medical	Yeast recombinant collagen gel	II	
Chuangming Medical	Yeast recombinant collagen gel dressing	II	
Chuangming Medical	Yeast recombinant collagen repair dressing	II	
Chuangming Medical	Yeast recombinant collagen dressing	II	
Chuangming Medical	Yeast recombinant collagen repair dressing	II	
Jinbo Biomedicine	Collagen dressing (hemorrhoid type)	II	Hemorrhoids
Chuangjian Medical	Yeast recombinant collagen wound gel dressing	II	
Chuangming Medical	Yeast recombinant collagen wound gel	II	
Chuangming Medical	Yeast recombinant collagen oral mucosa repair solution	II	Oral mucositis and oral ulcer
Chuangjian Medical	Yeast recombinant collagen oral mucosa repair solution	II	
Juzi Biology	Human like collagen oral mucosa repair fluid	II	
Chuangming Medical	Yeast recombinant collagen scar gel	II	Scar
Juzi Biology	Human like collagen scar repair silicone gel	II	
Chuangjian Medical	Yeast recombinant collagen scar gel	II	
Juzi Biology	Human like collagen nasal mucosa repair gel	II	Rhinallergosis
Juzi Biology	Carbomer like human collagen gynecological gel	II	Colpitis
HP Biology	Recombinant human collagen vaginal gel	II	
Miaote Pharmaceutical	Type III collagen vaginal gel	II	
Chuangming Medical	Yeast recombinant collagen gynecological gel dressing	II	

构建和调试优化突破自然进化的限制，实现人工设计指导下胶原蛋白的定量可控表达、功能定向强化以及规模化生产值得期待。

与大多数仿生材料的低生物活性相比，胶原蛋白具有良好的生物降解性和生物相容性，以及足够的可塑性，被广泛应用于食品、医疗用品、3D 打印、化妆品等多个行业，且全球胶原蛋白市场仍在持续增长。然而，胶原蛋白是亲水性的，机械强度差，需要进行改性程序以改善最终应用的物理化学性能。基于胶原蛋白的仿生材料可以通过多种材料进行改性，以提高其生物学性能和机械性能。目前基于胶原蛋白的仿生材料通常为柔性水凝胶和刚性支架。虽然，这些胶原复合材料已经在体外用于多种组织修复，但是仍然缺乏完整的体内实验来验证这些材料的实用性。此外，要制造能够满足所有所需性能(包括孔隙率、孔径、生物相容性、机械完整性、结构稳定性)的复合材料用于组织修复与再生仍然是一个挑战。但随着生物打印技术、组织工程和仿生技术的发展，复合胶原基材料在组织工程领域的应用有望取得突破。

此外，胶原蛋白作为皮肤中重要成分，凭借其良好的支撑、修复、保湿、美白等性能，在注射填充材料、功效性敷料、功效性护肤品及一般护肤品领域中扮演一个重要材料角色。其中，胶原蛋白有望凭借其支撑填充、修复、保湿及美白四重功效成为填充领域的重要材料之一。目前，各胶原蛋白企业纷纷完善下游胶原蛋白产品布局，扩充产品管线和拓展品类，使胶原蛋白逐步从医用场景走向多场景，实现“严肃医疗-消费医疗-日化”全覆盖。

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