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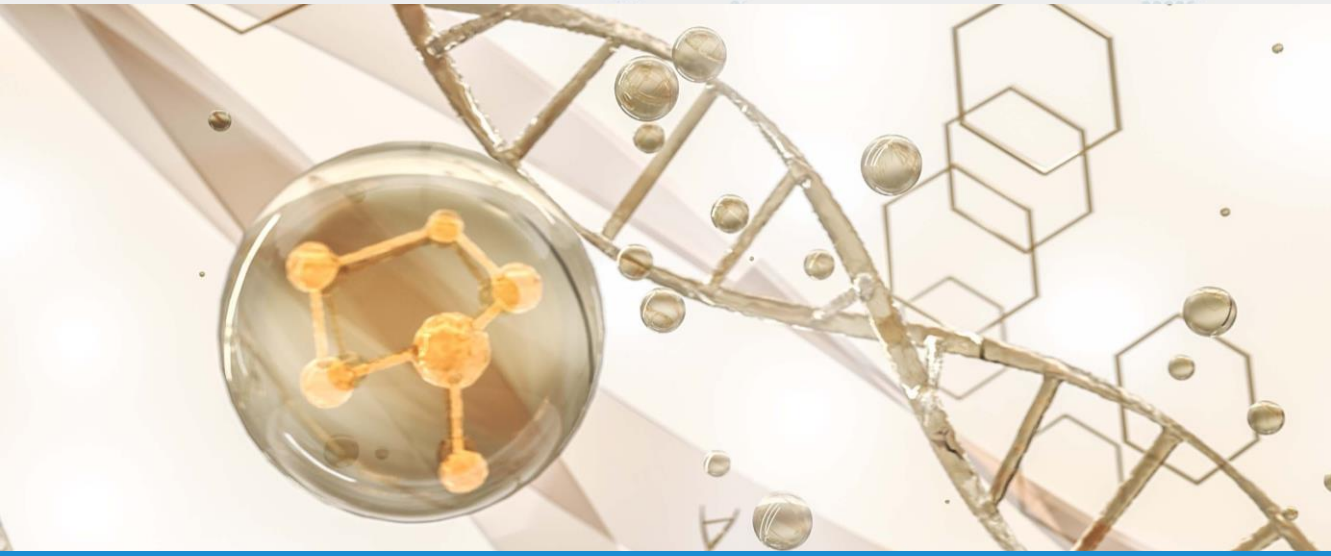

Recombinant Type III Triple-Helix Collagen Whitepaper

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Introduction

Collagen is an essential structural element of the extracellular matrix, found in all tissues and organs.It does not only provide strength, durability, and elasticity to tissues but also plays a vital role in numerous biological processes. Among the various types of collagen, Type III collagen—one of the first to be identified and utilized—has attracted growing market interest.

The triple-helix structure of collagen is closely related to its biological functions. This white book investigates the significance and potential applications of Type III collagen. It focuses on the triple-helix structure of Type III collagen, offering a comprehensive discussion of its structure, functions, production methods, and future application prospects.

- To date, 28 different types of collagen have been identified and can be classified into different categories based on their functions. Type III collagen is a fibrillar type consisting of three identical polypeptide chains that intertwine to form a right-handed superhelix, commonly referred to as the triple-helix structure.
- This white book explores the relationship between the structure and function of Type III collagen, emphasizing the essential role of its fibrillar network in preserving the structural integrity of skin and tissues. It also discusses its role in the formation and function of other collagens, facilitating cell adhesion, migration, and proliferation, supporting wound healing, and inhibiting tumor cell growth.
- In recent years, recombinant collagen has attracted significant attention due to its low immunogenicity and high safety. Using recombinant Type III collagen as an example, this white paper thoroughly analyzes the characteristics and advantages of producing recombinant Type III triple-helix collagen through synthetic biology techniques. It also addresses the key control points in the production process that are essential for forming the triple-helix structure.
- The strong functionality of Type III triple-helix collagen lays a solid theoretical foundation for its wide-ranging applications across various fields. Recombinant Type III collagen has already been found useful in serious medical treatments, aesthetic medicine, functional skincare, and health foods. This white paper offers a comprehensive analysis of its current applications, market trends, and the significant market potential of recombinant Type III triple-helix collagen in multiple industries.
- We hope this white book will be a valuable resource for professionals and consumers in related fields, further advancing scientific research and clinical development of recombinant Type III triple-helix collagen. We look forward to collaborating with our industry peers to embark on a new chapter in the research and application of recombinant Type III collagen.

Editorial Board

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Foreword

Guangdong Jinmeiji Group is an enterprise that integrates drug production and R&D, drug distribution and sales, as well as medical services and healthcare within its industrial park. With 29 years of history, the company operates four GMP production lines, several GSP-certified companies, and general hospitals. Drawing on extensive experience in pharmaceutical research, production, management, and marketing, along with a robust quality management system, its subsidiary Everon Healthcare specializes in leading and uniquely crafted recombinant humanized type III collagen technology.

This white book adopts a rigorous scientific approach and a broad industry perspective. It starts by exploring foundational research on collagen, offering a thorough explanation of its definition, classification, structural characteristics, and vital role within the human body. It delves particularly into the discovery and structural features of Type III collagen, highlighting its phenomenal contributions to maintaining skin stability, tissue elasticity, and wound healing. At the same time, we highlight the innovative advancements in recombinant Type III triple-helix collagen technology. This includes its design principles, production methods, and bioactivity assessment, demonstrating the significant advantages of this technology in enhancing purity, safety, and adaptability.

Notably, Everon Healthcare has successfully applied recombinant Type III triple-helix collagen, offering a glimpse into the extensive application potential of this technology. Its prospects span multiple fields, including medical specialties, medical aesthetics, and functional skincare industries. With a fully humanized design, AI-assisted optimization, and an exceptionally high-temperature stable triple-helix structure, Everon Healthcare is at the forefront of industry innovation. These advancements provide patients with safer and more effective treatment options, setting a new standard in the field. It provides more possibilities for those pursuing beauty and health, making beauty more efficient and trustworthy. This trusted ingredient marks the beginning of a new era in humanity's pursuit of beauty, offering unlimited benefits and opportunities to explore aesthetics further.

Looking ahead, the future of recombinant Type III triple-helix collagen technology holds immense promise. As research advances and technology evolves, we are confident that this innovation will showcase its unique value across an even wider range of fields. It has the potential to drive breakthroughs in the medical aesthetics industry and contribute significantly to human health. At Everon Healthcare, we believe this triple-helix ingredient marks the starting point of our journey in synthetic biology, and our goal remains to deliver more groundbreaking raw material technologies to benefit people. Together, let us explore the untapped potential of this field and usher in a new era of health and beauty.

As the General Manager of Everon Healthcare, I am truly honored to be part of this meaningful endeavor. As a member of the Jinmeiji Group, I aspire for our contributions in medicine and pharmaceuticals to explore beyond saving lives and alleviating pain, but also to help sustain and strengthen people’s enduring pursuit of beauty. Medical aesthetics, with safe and reliable ingredients, not only treat physical illnesses but also foster confidence and well-being in our society, propelling human progress in beauty and relaxation. I am confident that the release of the "2024 White Book on Recombinant Type III triple-helix Collagen" will become a significant milestone in advancing this field. Let this be the foundation for continued dedication and progress, as we jointly create a healthier and more beautiful in the future.

—— Aries Yang, Chief Executive Officer of Everon Healthcare

Foreword

In this fast-evolving age of biotechnology, I am very fortunate to serve as the Chief Scientist at Everon Healthcare and the founder of Liying Biotech, where I lead a team dedicated to exploring the complexities of recombinant collagen. Collagen, as one of the most critical structural proteins in living organisms, has consistently inspired our passion for research due to its distinctive structure and functions. In this white book, we will be focusing on the next generation of recombinant Type III collagen technology, to explore its immense potential across multiple fields and turn it into practical applications.

Recombinant Type III collagen is a biomaterial with significant potential across various practical fields. Its distinct structural and functional properties make it especially promising in areas like medical aesthetics and biomaterials. Our vision is to advance the next generation of recombinant Type III collagen into a highly efficient, safe, and widely accessible biomaterial. Through ongoing technological innovation and research investment, we aim to contribute to both human health and beauty.

Traditionally, collagen is primarily extracted from animal tissues. Although this method yields collagen with a natural triple-helix structure, it presents potential safety risks and immunogenicity concerns. To overcome these limitations, recombinant DNA technology enables us to produce recombinant humanized Type III collagen, which offers significant safety advantages. However, the inability to fully replicate the natural triple-helix structure remains a significant challenge in the field of recombinant collagen. Leveraging Liying Biotech's expertise in synthetic biology and extensive experience in protein structure research, Everon Healthcare has partnered with Liying Biotech to carry out in-depth research and development of next-generation recombinant Type III collagen technology. This collaboration has allowed for a focused exploration of advanced solutions in the field. As a team with years of experience in protein structure analysis and design across top international laboratories, we have a deep understanding of the principle that " the protein structure defines its function." By leveraging the latest advancements in structural biology and AI-assisted computational systems, along with our dedication to excellence, we have accomplished several breakthroughs in this field. We are proud to be the first to industrialize recombinant humanized collagen featuring a stable triple-helix structure.

This white book aims to provide readers with a comprehensive perspective on the latest R&D, production technologies, application fields, and market prospects of recombinant Type III triple-helix collagen. We start this book by outlining the basic knowledge of collagen, including its definition, classification, structure, functions, and synthesis mechanisms, with a special emphasis on the unique properties of Type III collagen. We then examine the production process, key technologies, and quality control standards for recombinant Type III collagen. Additionally, we highlight its extensive applications in serious medical treatments, medical aesthetics, functional skincare, and other consumer sectors.

In the section covering production processes and key technologies, we explore sequence design and optimization, as well as the selection and enhancement of expression systems. We also discuss critical stages such as expression, purification, and detection, and highlight the distinctive features and advantages of its triple-helix structure. We also provide a comprehensive analysis of the recombinant collagen market, project future trends, and highlight Everon Healthcare's innovative practices and case studies in this area. We believe this white book will provide valuable insights for medical professionals, biotechnology researchers, industry analysts, and investors interested in collagen applications.

We hope this white book serves as a valuable reference for researchers, professionals, and decision-makers in related fields. We aim to promote further research and development of recombinant Type III triple-helix collagen and facilitate its widespread application across various industries. Finally, we extend our gratitude to all individuals and organizations who contributed to the drafting and publication of this white book. We look forward to working together with our industry colleagues to advance collagen research and applications, making greater contributions to human health and beauty.

—— Ying Zhang, Chief Scientist of Everon Healthcare

Catalogue

Chapter 1: An Overview of Collagen

Chapter 2: Properties and Functions of Type III Collagen

Catalogue

Chapter 3: Recombinant Type III Triple-Helix Collagen

Chapter 4: Applications of Recombinant Type III Triple-Helix Collagen

Catalogue

Chapter 1

An Overview of Collagen

01

1.1 Definition and Function of Collagen

When three collagen protein chains form a triple-helix structure, they are referred to as “collagen”. As a key component of the extracellular matrix, it provides structural support, aids tissue repair, and assists in hemostasis.

1.1.1 Definition of Collagen

Collagen, also known as collagen peptide, refers to the triple-helix structure found in body tissues. It is a critical structural component of the extracellular matrix, present in tissues and organs such as the skin, bones, tendons, ligaments, and cartilage. Collagen provides strength, durability, and elasticity to tissues, and plays a key role in various biological interactions. It is found in the extracellular matrix of all multicellular organisms, including sponges, invertebrates, and vertebrates. The protein monomer that forms collagen fibers is called tropocollagen. Both tropocollagen and collagen are the natural forms of collagen, with collagen being a complex structure formed through the multi-level assembly of collagen molecules. Collagen typically consists of three peptide chains, known as α-chains. In some collagen molecules, the α-chains are identical, while in others, they are different. The chains are usually referred to as α1, α2, and α3 chains. For different types of collagens, Roman numerals in brackets are used for notation. For example, the α1 chain of type III collagen is denoted as α1(III), and type III collagen is represented as $[\alpha1(III)]_3$.

Collagen is defined as a structural protein in the extracellular matrix, with its molecules containing at least one domain or a region of triple-helix α-chains, known as the collagen domain. Each α-chain is a collagen protein molecule, and collagen itself is a right-handed triple-helix formed by three collagen protein molecules. For type III collagen, each collagen protein molecule has a molecular weight of approximately 138,000 Da.

Fig. Structure, Location, and Function of Collagen in the Human Body (Using Cartilage as an Example)

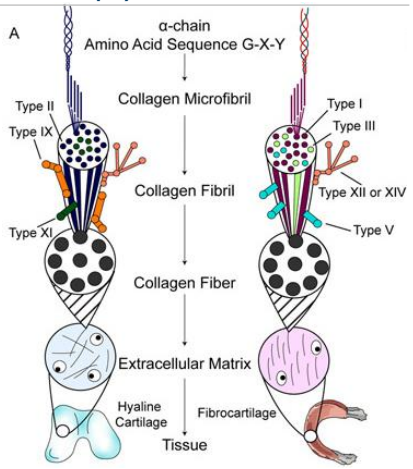


Figure Source: Bielajew BJ, Hu JC, Athanasiou KA. Collagen: quantification, biomechanics, and role of minor subtypes in cartilage. Nat Rev Mater. 2020 Oct;5(10):730-747. doi: 10.1038/s41578-020-0213-1. Epub 2020 Jul 20.

1.1.2 Functions of Collagen

Collagen, a natural high-molecular protein, plays a crucial role in the human body. It provides structural support, aids in tissue repair, and has hemostatic properties. Due to its essential role in human tissues and its significant advantages as a biomaterial, collagen-based tissue engineering and biomedical materials have achieved notable success in clinical practice and are widely recognized by both physicians and patients.

Fig. Primary Functions of Collagen in the Human Body

Hemostatic Properties

When the blood vessel endothelium is damaged, the underlying tissue, mainly collagen, becomes exposed. This triggers the local coagulation mechanism, leading to the formation of a blood clot composed of fibrin polymers. Collagen, working with platelets, forms a thrombus to control bleeding. Natural collagen aggregates are excellent hemostatic materials.

Low Immunogenicity

Collagen's highly repetitive structure gives it very low immunogenicity, meaning the body usually does not mount a chronic rejection response. Therefore, collagen-based allogeneic tissue devices can be implanted for long-term use in the human body.

Cellular Interactions

Collagen provides attachment and support for cell growth, guiding the proliferation, differentiation, and migration of epithelial and other cells. During embryonic development, wound healing, and tissue remodeling, collagen interacts with cells to facilitate their functions. It is essential in promoting and inducing epithelial formation.

Biocompatibility

Collagen forms the scaffold of the extracellular matrix. Its unique triple-helix structure provides excellent biocompatibility, enabling it to act as a scaffold for new tissue to be absorbed and integrated by the host.

Biodegradability

Collagen undergoes peptide bond cleavage and helix disruption under collagenase action, leading to hydrolysis. At body temperature, the hydrolyzed fragments can spontaneously denature, then be further broken down by other enzymes into oligopeptides or amino acids. These products are eventually utilized by the body or excreted.

Sources: Public information, literature search, and Frost & Sullivan analysis.

1.2 Classification of Collagen

Currently, 28 types of collagens have been identified and classified into four main categories based on function: fibrillar collagens, FACIT, reticular collagens, and MACITs.

1.2.1 Classification of Collagen

Collagen is a diverse protein family, varying in genetics and molecular structure across species and tissues. Collagen in different populations and tissues has unique chemical compositions or conformations. Each α-chain in a tropocollagen molecule can be classified into several subtypes, and each α-chain is encoded by a specific gene. In theory, over 20 α-chains can combine to form more than 1,000 types of collagens, and to date, 28 types have been identified.

Collagen Classification

Collagen can be classified into four major types based on its primary structure, the length of the triple-helix domain, molecular weight, interruptions in the triple-helix, and the size and shape of the terminal domains.

Fig. Four Major Types of Collagen

	Type	Features
Fibrillar Collagen	I、II、III、V、XI、XXIV、XXVII	Types I, II, and III collagen are most prevalent in vertebrates. Types V and XI are less common but assist in the assembly of Types I, II, and III. Types XXIV and XXVII have shorter interrupted triple-helix domains. Types III, V, and XI collagen retain their C-terminal peptide and part of the N-terminal peptide domain after processing.
FACIT *(Fibril-Associated Collagens with Interrupted Triple Helices)*	IX、XII、XIV、XVI、XIX、XXII	FACIT collagens do not form fibrillar structures but interact with fibrillar collagens, regulating their formation, size, and synthesis in the extracellular matrix.
Reticular Collagen	IV、VII、XXVIII	Type IV collagen forms a fibrous network. Type VII collagen assembles into anchoring fibers that link the epidermis to the dermis.
MACITs *(Membrane-Associated Collagens with Interrupted Triple Helices)*	XIII、XXIII、XXV	Types XIII, XXIII, and XXV collagen are Type II transmembrane proteins, consisting of a hydrophobic transmembrane domain, a short N-terminal cytoplasmic domain, and three extracellular collagen domains.

Type of Collagen

Collagen types are classified based on their order of discovery: Type I, Type II, Type III, and so on. The most common types of collagens in the human body are: ① Type I collagen (most abundant, primarily found in adult skin and bone); ② Type II collagen (mainly present in cartilage); ③ Type III collagen (predominantly found in infant skin, blood vessels, endothelium, intestines, and stomach organs); ④ Type IV collagen (mainly located in the basement membranes of various tissues and organs, as well as in the placenta and lens).

Fig. Types of Collagen in the Human Body (1/2)

Structural Type	Type	Gene Symbol	Chain Composition	Supramolecular Structure	Tissue Distribution
Fibrillar Collagens	I	COL1A1	$[\alpha1(I)]_2\alpha2(I)$	67 nm Banding Pattern Fibrils	Bone, Tendons, Dermis, Cornea
		COL1A2	$[\alpha1(I)]_3$		
	II	COL2A1	$[\alpha1(II)]_3$		Cartilage, Vitreous Body of Eye
	III	COL3A1	$[\alpha1(III)]_3$		Dermis, Aorta, Uterus, Intestines
	V	COL5A1	$[\alpha1(V)]_2\alpha2(V)$	9 nm Banding Pattern Fibrils	Placenta, Bone, Dermis, Cornea
		COL5A2	$[\alpha1(V)\alpha2(V)\alpha3(V)]$		
		COL5A3	$[\alpha1(V)]_3$		
	XI	COL11A1	$[\alpha1(XI)\alpha2(XI)\alpha3(XI)]$	Fine Fibers (Similar to Type V Collagen Fibrils)	Cartilage, Intervertebral Discs
		COL11A2			
	XXIV	COL24A1	$[\alpha1(XXIV)]_3$	Presumed Formation of Homotrimeric Fibrils	Cornea, Bone
	XXVII	COL27A1	$[\alpha1(XXVII)]_3$		Cartilage, Eye, Ear, Lung, Colon

Sources: Public information, literature search, and Frost & Sullivan analysis.

1.2 Classification of Collagen

Currently, 28 types of collagens have been identified and classified into four main categories based on function: fibrillar collagens, FACIT, reticular collagens, and MACITs.

Fig. Types of Collagen in the Human Body (2/2)

Structural Type	Type	Gene Symbol	Chain Composition	Supramolecular Structure	Tissue Distribution
FACIT	IX	COL9A1 COL9A2 COL9A3	[a1(IX)a2(IX) a3(IX)]	Binding with Type II Collagen Fibers	Cartilage, Vitreous Body
	XII	COL12A1	$[a1(XII)]_3$	Binding with Type I Collagen Fibers	Dermis, Tendons, Cartilage
	XIV	COL14A1	$[a1(XIV)]_3$	Binding with Type I and Type II Collagen Fibers	
	XVI	COL16A1	a1(XVI)	Binding with Fibronectin-1 and D-Band Type II Collagen Fibers	Heart, Kidney, Smooth Muscle
	XIX	COL19A1	a1(XIX) $[a1(XIX)]_2$ $[a1(XIX)]_3$ $[a1(XIX)]_4$ $[a1(XIX)]_5$ $[a1(XIX)]_6$	N-terminus Oligomerization	Basement Membrane of Differentiated Muscle Cells
	XX	COL20A1	a1(XX)	Presumed Binding with Collagen Fibers	Corneal Epithelium
	XXII	COL22A1	a1(XXII)	Presumed Binding with Basement Membrane Components	Tendon Insertion Sites, Joint Cartilage-Synovial Interface, Hair Follicles
Reticular Collagen	IV	COL4A1 COL4A2 COL4A3 COL4A4 COL4A5	$[a1(IV)]_2a2(IV)$ $[a1(IV)]_2a3(IV)$ $[a1(IV)]_2a4(IV)$ $[a1(IV)]_2a5(IV)$ $[a1(IV)]_2a6(IV)$	Non-fibrillar Network	Basement Membrane
MACITs	VII	COL7A1	$[a1(VII)]_3$	Anchor Fibers	Skin, Amnion, Mucosal Epithelium
	XVIII	COL18A1	a1(XVIII)	Highly Vascularized Tissue Expression	Lung, Liver, Kidney
	XIII	COL13A1	a1(XIII)	Protects Skin	Endothelial Cells, Epidermis
	XXIII	COL23A1	a1(XXIII)	Unknown	Tumors (Prostate)
	XXV	COL25A1	a1(XXV)	Unknown	Alzheimer's Disease Amyloid Plaques
	XXVI	COL26A1	a1(XXVI)	Unknown	Testis, Ovary
Uncategorised collagen	XVIII	COL18A1	a1(XVIII)	Highly Vascularized Tissue Expression	Lung, Liver, Kidney
	XV	COL15A1	a1(XV)	Involved in Expression	Fibroblasts, Endometrium
	XXVIII	COL28A1	a1(XXVIII)	Unknown	Basement Membrane, Peripheral Nervous System

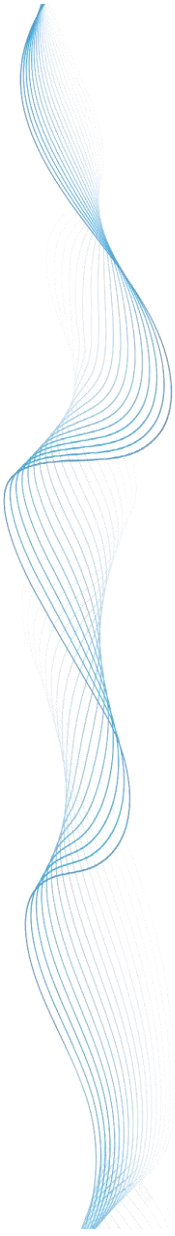
Sources: Public information, literature search, and Frost & Sullivan analysis.

1.3 Introduction to Type III Collagen

In 1971, Miller et al. discovered type III collagen. Since then, research on its structure, distribution, function, and preparation has advanced, and recombinant collagen technology has broadened its applications.

1.3.1 Introduction to Type III Collagen

Type III collagen was first identified and described by Miller et al. in 1971. It is an extracellular matrix protein synthesized by cells in a precursor form known as pre-procollagen. Type III collagen serves as a key structural component in hollow organs such as large blood vessels, the uterus, and the intestines.



1971

Miller et al. were the first to identify and describe type III collagen.

1989

Tromp, G. et al. discovered a link between type III collagen gene mutations and Ehlers-Danlos Syndrome (EDS). These mutations disrupt the normal structure and function of collagen, causing the clinical symptoms seen in EDS.

1980s ~ 1990s

The development of genetic engineering enabled the production of collagen through recombinant DNA technology, laying the foundation for its large-scale production and application.

1991

Scientists successfully extracted type III collagen from human bone, overturning the previous belief that it was absent in bone. This discovery laid the theoretical foundation for its future applications in orthopedics.

~1994

By utilizing point mutation techniques, foreign scientists were the first to unravel the atomic-level structure of collagen. They discovered that collagen consists of three tightly interwoven protein chains, forming a right-handed triple-helix structure.

1997

Klug and Cummings conducted an in-depth study on the supramolecular structure of collagen, focusing on the unique characteristics of its triple-helix formation. They also explored how collagen assembles within tissues, particularly highlighting the 67nm periodic banding pattern found in various collagen types, such as Type I, II, and III.

2005

The granting of China’s first patent for recombinant collagen marks a significant milestone in the use of genetic engineering to produce collagen. This milestone provides a strong foundation for future industrialization and applied research efforts.

2021

China's first humanized type III collagen for cosmetic injection was approved and rapidly gained market recognition, marking the beginning of rapid development in the research and approval of recombinant collagen injectables for cosmetic purposes.

Sources: Public information, literature search, and Frost & Sullivan analysis.

1.3 Introduction to Type III Collagen

Type III collagen has a stable triple-helix conformation, determined by its specific amino acid sequence, which plays a crucial role in its biological functions.

1.3.2 Structure of Type III Collagen

The biological function of collagen largely depends on its spatial structure. Different types of collagen have distinct physiological functions and structures. For example, collagen in tendons is a high-strength protein with a highly asymmetric structure, while in skin, it forms soft fibers. In teeth and bones, collagen's hard portions contain calcium phosphate polymers, and in the cornea, collagen is crystal clear. It is widely accepted that only proteins with a tertiary structure or higher have physiological functions, and collagen possesses a complete quaternary structure.

As shown in the figure, Type III collagen is synthesized intracellularly as procollagen. After the signal peptide of 24 amino acids is cleaved, the procollagen chain is formed. Three α-chains assemble into a homotrimer, or Type III procollagen molecule. Proteases remove the large globular domains at the carboxy terminus (C) and Amino terminus (N) ends to form Type III collagen. Several co-translational and post-translational modifications also take place.

Fig. Structural Domains of Type III Procollagen Molecule

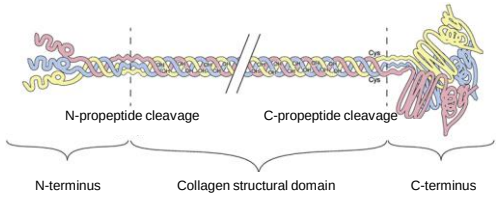


Figure Source: Kuivaniemi H, Tromp G. Type III collagen (COL3A1): Gene and protein structure, tissue distribution, and associated diseases. Gene. 2019 Jul 30;707:151-171. doi: 10.1016/j.gene.2019.05.003. Epub 2019 May 7. PMID: 31075413; PMCID: PMC6579750.

Primary Structure

The primary structure refers to the amino acid sequence of the polypeptide chain, including the positions of disulfide bonds. In collagen, the triple-helical region formed by α-chains is known as the Collagen structural domain, with each collagen molecule containing at least one. Mature Type III collagen consists of 1,068 amino acids per chain, and three chains form a triple-helix with a molecular weight of nearly 400 kDa. The Type III collagen sequence includes a repeated Gly-X-Y pattern, appearing 343 times. While X and Y can vary, X is typically proline (Pro), and Y is often hydroxyproline (Hyp) or hydroxylysine (Hyl). These tripeptide repeats are essential for maintaining the structure of collagen.

Secondary Structure

The secondary structure refers to the specific helical or folded configuration of the polypeptide chain backbone, forming a particular spatial arrangement or an unordered structure. Collagen's secondary structure takes the form of a left-handed α-helix, built by polyproline II (PP II) chains. Each PP II chain contains approximately 3.3 amino acid residues per turn, with the projection length of each residue along the helical axis being 0.286 nm and a helical pitch of 0.858 nm.

Tertiary structure

Three PP II chains can further assemble into collagen's characteristic triple-helix structure. The repetitive "Gly-X-Y" sequence in the α-chain creates steric repulsion between proline at X and hydroxyproline at Y, facilitating helix formation. Hydrogen bonds between the amide hydrogen and carbonyl oxygen of adjacent residues, parallel to the helical axis, further stabilize the structure. A typical collagen molecule is made up of three left-handed α-chains that intertwine into a left-handed superhelix. The middle section forms a continuous triple-helix, while the terminal regions are disordered, forming terminal peptide structures. Collagen's crystal structure reveals a highly organized water network critical to stabilizing the molecular conformation and triple-helix interactions. Techniques like NMR have advanced our understanding of sequence-dependent triple-helix formation, proving its importance in biological interactions and structural functions.

Quaternary Structure

Collagen's quaternary structure typically involves multiple peptide chains, each with its own distinct tertiary structure. These chains, called subunits, generally lack function when isolated. Arranged in a specific pattern, they form a higher-order protein with a three-dimensional structure, resulting in collagen molecules with very short non-triple helical regions, known as terminal peptides. In fibrillar collagen, for instance, the molecules form insoluble fibers via intramolecular and intermolecular forces, making most collagens insoluble, rigid proteins.

Sources: Public information, literature search, and Frost & Sullivan analysis.

1.3 Introduction to Type III Collagen

Type III collagen biosynthesis in the human body involves both intracellular and extracellular processes, with multiple modifications required to form and stabilize its triple-helical structure and enable its functions.

1.3.3 Mechanism of Type III Collagen Formation in the Human Body

Type III collagen is synthesized intracellularly as pre-procollagen, undergoing multiple co-translational and post-translational modifications. The signal peptide is cleaved to generate procollagen molecules. Three identical Type III procollagen chains aggregate at the C-terminus and stabilize through disulfide bonds. Each chain folds into a left-handed helix, and the three chains then coil together to form a right-handed superhelix, or triple-helix.

Intracellular Synthesis Phase

In tissue cell nuclei, the genetic information for each collagen polypeptide chain is transcribed by messenger RNA (mRNA) onto ribosomes, where polypeptide chains of over 1,000 amino acid residues are synthesized. These chains are then transported to the endoplasmic reticulum (ER) for hydroxylation and glycosylation modifications.

- **Hydroxylation Modification:** In the ER, proline and lysine residues are hydroxylated by prolyl hydroxylase and lysyl hydroxylase. Hydroxylation plays an important role in stabilizing the triple-helix. Under-hydroxylated polypeptide chains cannot form a stable triple-helix at body temperature and therefore cannot be secreted extracellularly.
- **Glycosylation Modification:** In the ER, polypeptide chains are glycosylated by galactosyl transferase and glucosyl transferase, attaching sugar groups to hydroxylysine residues. This modification promotes the alignment of fibrils. After hydroxylation and glycosylation, soluble collagen proteins can form the triple-helical procollagen and be secreted extracellularly.
- **Proline Residues:** Of the 239 proline residues in the triple-helix domain, approximately 145 are hydroxylated by prolyl-4-hydroxylase to form 4-hydroxyproline.

Proline Residue Hydroxylation and Triple-Helix Formation

Proline hydroxylation results in the formation of either 4-hydroxyproline or 3-hydroxyproline, catalyzed by different enzymes. 4-Hydroxyproline is believed to stabilize the triple-helix structure, while the exact function of 3-hydroxyproline remains unclear, though it may be involved in interactions between triple-helix and supramolecular assembly.

- **Lysine Residues:** Some lysine residues are hydroxylated or glycosylated, while others, including hydroxylysine residues, undergo oxidative deamination catalyzed by lysyl oxidase.

After undergoing hydroxylation and glycosylation modifications, soluble collagen can form a triple-helical procollagen and be secreted extracellularly.

Extracellular Maturation Phase

After the formation of the triple-helix, some additional post-translational modifications continue to occur.

- **Terminal Cleavage:** The large globular domains at both the C-terminal and N-terminal of the molecule are cleaved by proteases, producing a triple-helix type III collagen monomer known as tropocollagen.

C-Propeptide and Triple-Helix Formation

The C-terminal propeptide plays a crucial role in the biosynthesis of fibrillar collagen. It guides chain selection, stabilizes associated α-chains through interchain disulfide bonds, and promotes the formation of the triple-helix. In Type III procollagen, the C-propeptide contains eight cysteine residues and one N-glycosylation site. Studies have confirmed that the C-propeptide forms the expected interchain and intrachain disulfide bonds. Additionally, research has revealed the crystal structure of the C-propeptide in human Type III procollagen. This finding elucidates the intricate structural mechanism by which procollagen chains are recognized during their intracellular trimerization.

- **Cross-Linking:** Certain lysine and hydroxylysine residues form cross-links, which further stabilize the structure.

Sources: Public information, literature search, and Frost & Sullivan analysis.

1.3 Introduction to Type III Collagen

The triple-helical structure endows type III collagen with numerous structural and physiological functions, making it the primary structural component of hollow organs and widely distributed in living organisms.

1.3.4 Distribution of Type III Collagen

Type III collagen is a key structural component of hollow organs such as large blood vessels, the uterus, and the intestines, which need to withstand stretching. It also coexists with type I collagen in many other tissues. Numerous studies have examined the distribution and function of type III collagen across different tissues and age groups in mice and/or humans.

◘ Distribution by Tissue Location

According to a 2014 study by Fagerberg et al., mRNA expression analysis was conducted using RNA sequencing from 27 different human organs and tissues of 95 individuals. The results show that COL3A1 mRNA is highly expressed in the gallbladder, placenta, bladder, and endometrium. Type III collagen has been detected throughout the cortex, with higher concentrations observed on the surface of Haversian canals and at the bone-periosteum interface.

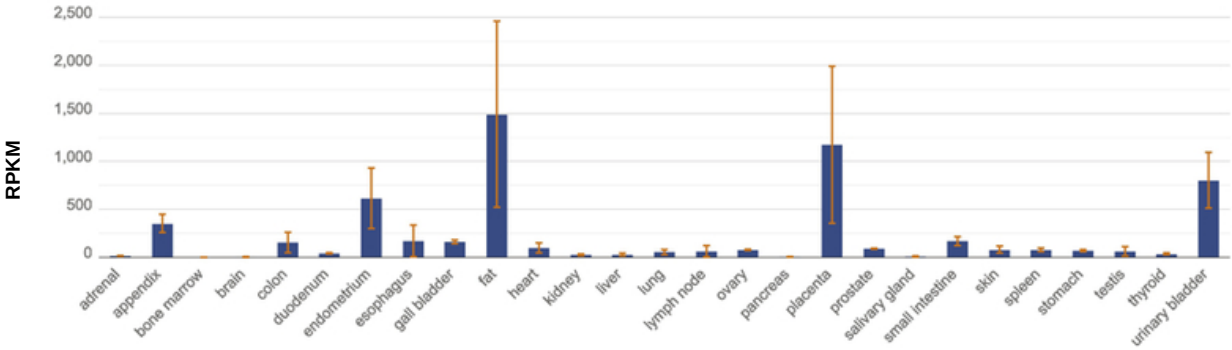
◘ Distribution by Age

Studies have shown that during embryonic development, type I and type III collagen are expressed in a coordinated manner. This is based on a comprehensive survey of mouse embryos at various developmental stages from E7.5 to E17.5 using in situ RNA hybridization techniques. In 1991, Keene et al. first extracted type III collagen from human bone and found it present in samples from donors of all ages (30 weeks to 80 years), indicating its presence throughout life.

◘ Other Distribution

In 1984, d'Ardenne et al. studied the expression of type III collagen in different benign and malignant tumors using immunostaining. All tumor samples showed a positive reaction to type III collagen. Among benign tumors, the highest expression levels of type III collagen were found in leiomyomas and giant cell tumors of the tendon sheath, while in malignant tumors, the highest levels were observed in leiomyosarcoma, fibrosarcoma, and sarcoma.

Fig. Distribution of Type III Collagen in Human Tissues and Organs



Source: Kuivaniemi H, Tromp G. Type III collagen (COL3A1): Gene and protein structure, tissue distribution, and associated diseases. Gene. 2019 Jul 30;707:151-171. doi: 10.1016/j.gene.2019.05.003. Epub 2019 May 7. PMID: 31075413; PMCID: PMC6579750.

Notes:

•Distribution of COL3A1 mRNA in human tissues is based on RNA sequencing data from Fagerberg et al. (2014).

•RPKM: Reads Per Kilobase per Million mapped reads, a standardized method for measuring relative gene expression levels.

Sources: Public information, literature search, and Frost & Sullivan analysis.

Chapter 2
Properties and Functions of Type III Collagen

02

2.1 Physicochemical Properties of Collagen

Different extraction methods and raw materials can impact the physicochemical properties of collagen. For instance, regarding thermal stability, collagen from terrestrial animals typically shows greater stability than that from aquatic animals.

2.1.1 Physicochemical Properties of Collagen

Natural collagen is widely found in biological organisms, and in the extraction and preparation process, mammals such as pigs and cows, as well as fish, serve as the primary sources.

The thermal stability of collagen

The performance of collagen from different sources in various application fields is largely influenced by its thermal stability. The thermal stability of collagen mainly depends on the content of proline and hydroxyproline within the molecular structure. These amino acids form pyrrolidine rings and hydrogen bonds, thereby enhancing the stability of the molecule. As the content of proline and hydroxyproline increases, along with a higher degree of cross-linking, the thermal stability of collagen improves accordingly.

Typically, collagen from terrestrial mammals, such as sheep bones, cowhide, pigskin, and rat-tail tendons, demonstrates higher thermal stability than collagen derived from aquatic animals, with a higher thermal denaturation temperature.

Fig. Content of Hyp and Pro in Collagen from Different Sources and Their Thermal Denaturation Temperatures

Source of Collagen	Amino Acid Content (%)		Thermal Denaturation Temperature (°C)
	Hydroxyproline (Hyp)	Proline (Pro)	
Sheep bones	10.14	11.95	38.9
Rat-tail tendons	6.85	11.10	37.0
Cowhide	9.40	12.10	36.3
Pigskin	9.70	12.30	35.8
Tilapia skin	9.05	12.64	35.0
Red snapper skin	8.72	11.79	34.0
Pollock skin	6.90	11.50	24.6
Deep-sea red salmon skin	5.60	9.10	15.9

Relationship Between Collagen Properties and Extraction Methods

Acid, alkaline, and enzymatic extraction methods for collagen all involve extended soaking in their respective solutions to remove impurity proteins and extract the collagen. The extracted collagen is then purified through salting out and prolonged dialysis. Prolonged exposure to alkaline, acidic, or saline chemical reagents may have varying effects on the chemical properties of collagen.

Research has shown that extraction time significantly affects collagen properties. For example, studies by LIN et al. indicate that the fibrillogenesis ability of bird claw collagen decreases with extended extraction time, and collagen subjected to prolonged extraction exhibits a lower thermal denaturation temperature compared to that extracted over shorter periods. Research by ZEUGOLIS et al. found that different extraction methods affect the viscosity and solubility of collagen. For instance, collagen extracted from bovine Achilles tendon using acetic acid has higher viscosity, whereas pepsin-extracted collagen shows significantly improved solubility in acidic conditions. Additionally, studies by YOSHIMURA et al. demonstrate that alkaline solution treatment disrupts the covalent bonds in the terminal peptides of collagen molecules, reducing intermolecular covalent cross-linking and thereby lowering its thermal stability. These studies highlight that extraction conditions and duration have a crucial impact on the final properties of collagen.

Sources: Public information, literature search, and Frost & Sullivan analysis.

2.2 Functions of Type III Collagen

Type III collagen is a protein widely distributed in human skin, fascia, tendons, and other tissues, playing a crucial role in biological functions and applications.

2.2.1 Type III Collagen in Maintaining Skin and Tissue Elasticity and Stability

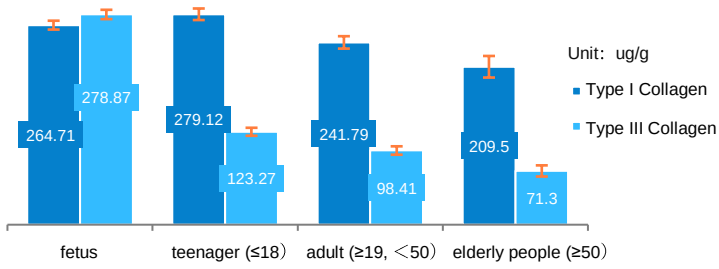
Collagen makes up 70% of the skin's composition and plays a crucial role in facilitating limb movement. It not only provides necessary protection but also gives the skin moderate elasticity and firmness, allowing for flexible movement and good elasticity when jumping. As a fundamental element for maintaining the elasticity of skin and muscles, collagen aids in connecting muscle cells, enhancing their elasticity and appearance.While muscle fibers and myosin are the main components of muscles, collagen serves as the adhesive between cells. The three-dimensional framework formed by collagen molecules helps maintain proper body posture and ensures adequate flexibility.

Type III collagen, the second most abundant collagen in the human body, is a critical structural component of fibrous collagen tissues. It is predominantly found in tissues such as blood vessels, internal organs, and muscles, where it plays a vital role in integrating and repairing the extracellular matrix. Within blood vessels, Type III collagen forms a dense network of fibers that supports and strengthens the vascular walls, maintaining their elasticity and stability, allowing them to withstand the pressure and impact of blood flow. In internal organs like the liver, lungs, and kidneys, Type III collagen offers structural support and protection, ensuring these organs function properly.

Skin Aging

- **Natural Aging:** As one ages, the amount of Type I and Type III collagen in the skin gradually decreases, with the ratio of Type I to Type III collagen increasing. After birth, the degradation of Type III collagen exceeds its synthesis, leading to its gradual loss. Type I collagen synthesis surpasses degradation before the age of 8, but this trend reverses during adolescence.

Fig. Content of Type I and Type III Collagen in Normal Human Skin (Mean ± SD)



- **Photoaging:** Ultraviolet (UV) radiation significantly contributes to skin photoaging, which is clinically observed as rough, thickened skin and dark wrinkles in areas frequently exposed to the sun. These regions may also exhibit hyperpigmentation, visible blood vessels, and a dull complexion. UV exposure can, in some cases, elevate the risk of skin cancer. Photoaging is characterized by increased activity of reactive oxygen species (ROS), matrix metalloproteinases (MMPs), and collagen degradation, which disrupts the extracellular matrix balance and decreases overall collagen levels in the body. Once lost, collagen cannot be regenerated. Without timely replenishment, the skin will progressively develop wrinkles, lose elasticity, and sag. Collagen in the dermis plays a crucial role in protecting skin cells from UV damage.

Fragility and Rupture of Blood Vessels and Tissues

- **Ehlers-Danlos Syndrome (EDS):** Mutations in the COL3A1 gene can lead to Ehlers-Danlos syndrome (EDS), a rare and potentially life-threatening genetic disorder. In EDS patients, mutations in the COL3A1 gene impair the synthesis of Type III collagen, which compromises the integrity and function of hollow organs such as blood vessels, the uterus, and intestines, increasing the risk of severe complications such as aneurysms, uterine rupture, and intestinal rupture.

- **Other Diseases:** Since Type III collagen is closely associated with vascular integrity, other phenotypes related to the COL3A1 gene include brain abnormalities characterized by frontal and parietal polymicrogyria and various fibrotic diseases. In these diseases, increased levels of Type III collagen have been found in multiple organs, leading to severe brain malformations that affect normal brain development or cause structural and functional changes in other organs.

Sources: Public information, literature search, and Frost & Sullivan analysis.

2.2 Functions of Type III Collagen

Research indicates that Type III collagen regulates collagen fiber diameter and participates in collagen fiber cross-linking through interactions with other types of collagen.

2.2.2 Involvement of Type III Collagen in the Formation and Function of Other Collagens

Regulating Collagen Fiber Diameter: ***Type III collagen, in conjunction with Type I collagen, forms heterotypic Type I/III collagen fibers, controlling the diameter of collagen fibers.***

Some processed Type III procollagen (i.e., pN-collagen III) can modulate the diameter of collagen fibers formed by Type I collagen by forming covalent polymers with them. pN-collagen III inhibits the rate at which Type I collagen assembles into fibers and reduces the amount of Type I collagen incorporated into the fibers.

Studies have shown that a reduction in Type III collagen leads to thickening and increased heterogeneity of collagen fibers in articular cartilage, indicating that the growth of fibrils is impaired in the absence of Type III collagen.

Involvement in Collagen Fiber Cross-Linking: ***Type III collagen molecules covalently cross-link with Type I and Type II collagens, among others***

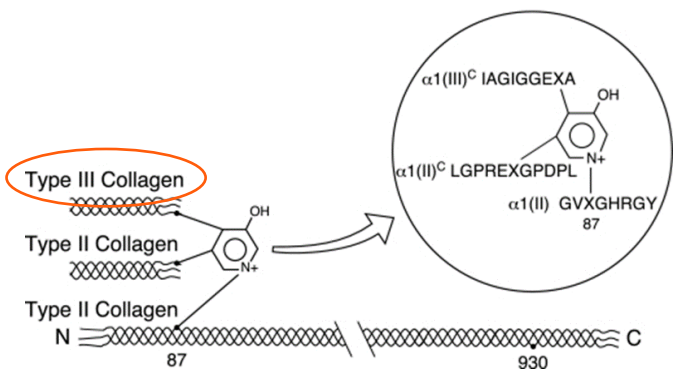
Type I, II, III, V, XI, and IX collagens create covalent enzyme-mediated cross-links, which are essential for the strengthening and maturation of the collagen network. This cross-linking process is vital for the tensile properties of collagenous tissues, with significant variation in cross-linking levels across different connective tissues.

Research has isolated peptides with intermolecular cross-links from insoluble matrices in human smooth muscle tumors and bovine aortas. These studies demonstrated that Type I and Type III collagen molecules are present within the same collagen fibers, linked via Out-of-D-phase (OD) displacement through intermolecular cross-linking.

- OD displacement describes the staggered arrangement of adjacent molecules during the assembly of collagen fibers. Collagen fibers are structured with molecules in a triple helix formation, where each helix is known as a monomer. During fiber formation, these monomers are arranged in a staggered manner to enhance the fibers' overall stability and mechanical properties.

Furthermore, other studies have shown that Type III collagen molecules, with unprocessed N-propeptides, exist as covalent cross-linked polymers in the extracellular matrix of adult human and bovine joint cartilage. These Type III collagen molecules are extensively cross-linked with Type II collagen. As a covalent modifier, Type III collagen may contribute to the repair of matrix damage by adding extra cohesion to the damaged Type II collagen fiber network, thereby reinforcing the structural stability of this network.

Fig. Structure of heterotypic II/III collagen cross-linked peptides purified from human articular cartilage



Source: Wu JJ, Weis MA, Kim LS, Eyre DR. Type III collagen, a fibril network modifier in articular cartilage. J Biol Chem. 2010 Jun 11;285(24):18537-44. doi: 10.1074/jbc.M110.112904. Epub 2010 Apr 19. PMID: 20404341; PMCID: PMC2881779.

Sources: Public information, literature search, and Frost & Sullivan analysis.

2.2 Functions of Type III Collagen

The sequence of Type III collagen includes receptor recognition sites on the cell surface, enabling processes like cell adhesion, migration, and proliferation through interactions with integrins and other molecules.

2.2.3 Type III Triple-Helix Collagen Promotes Cell Adhesion, Migration, and Proliferation

Integrins are heterodimeric transmembrane receptors that mediate cell adhesion. Through their extracellular head domains, most integrins interact with extracellular matrix glycoproteins like laminins and collagen in basement membranes, or with connective tissue components such as fibronectin. Certain integrins also bind to counter-receptors on neighboring cells, bacterial polysaccharides, or viral capsid proteins. These interactions enable integrins to mediate stable adhesion to the basement membrane and support extracellular matrix formation, as well as cell migration. Additionally, they promote platelet aggregation, establish intercellular connections within the immune system, and facilitate the entry of bacteria and viruses during infections. In addition, integrin-mediated adhesion regulates signaling cascades that control cell movement, survival, proliferation, and differentiation.

The sequence of Type III collagen contains key sites for promoting cell adhesion and signal transduction, such as GER or GEK integrin recognition motifs. These sites are located in the C-terminal region of procollagen III and exhibit a high affinity for integrin binding.

Promotion of Cell Adhesion

Researchers assessed the cell adhesion activity of recombinant Type III Triple-Helix collagen by conducting experiments on cultured NIH/3T3 cells. Fluorescence methods were used to detect adhesion activity, with various experimental groups, including single-chain collagen, triple-helix collagen, control, and blank groups. After a period of cultivation, cell adhesion rates were calculated to indicate the adhesive activity of the collagen samples. A higher adhesion rate signified more cells adhered to the protein, demonstrating stronger adhesion activity.

The results indicated that Type III collagen enhances cell adhesion, with recombinant Type III triple-helix collagen showing greater adhesion activity compared to the single-chain collagen and control groups. This suggests that recombinant Type III triple-helix collagen can promote rapid cell attachment or adhesion to the extracellular matrix, aiding in the creation of a more favorable extracellular environment.

Promotion of Cell Migration

Researchers cultured healthy BALB/3T3 cells using purified lyophilized recombinant Type III triple-helix collagen, control Type III single-chain collagen, and bovine serum albumin. Samples were collected, photographed, and analyzed at different time intervals to calculate migration rates. In this experimental system, cell migration activity effectively reflected the biological activity of the collagen; a higher migration rate and faster movement indicated greater biological activity.

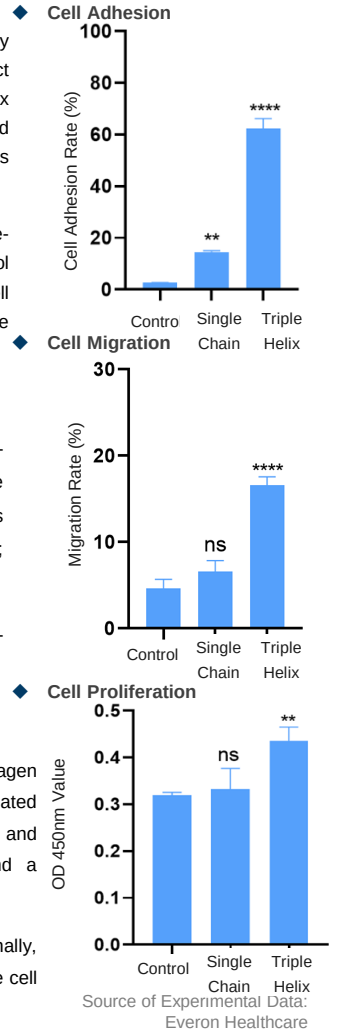
The results showed that Type III collagen promotes cell migration, with recombinant Type III triple-helix collagen exhibiting superior migration activity compared to Type III single-chain collagen.

Promotion of Cell Proliferation

Researchers examined the effects of recombinant Type III triple-helix collagen and single-chain collagen on the viability of mouse embryonic fibroblast cells (3T3) by measuring absorbance. They evaluated cytotoxicity and fibroblast proliferation activity. Cells in the exponential growth phase were collected and cultured with either recombinant Type III triple-helix collagen or single-chain collagen, and a multifunctional microplate reader was used to assess the viability in each group.

The results demonstrated that Type III collagen enhances the proliferation of 3T3 cells. Additionally, recombinant Type III triple-helix collagen showed significant safety and a stronger ability to promote cell proliferation compared to single-chain collagen.

Fig, Recombinant type III collagen in vitro test



Source of Experimental Data: Everon Healthcare

Sources: Public information, literature search, and Frost & Sullivan analysis.

2.2 Functions of Type III Collagen

Wound healing involves four stages: hemostasis, inflammation, proliferation, and remodeling. Type III collagen plays a crucial role in each stage, ultimately leading to the restoration of normal physiological conditions in the wound.

2.2.4 Role of Type III Collagen in Helping Wound Healing

Wound healing is a complex process that typically includes four stages: hemostasis, inflammation, proliferation, and remodeling.

Hemo-stasis

Platelets are a vital component of blood. When blood vessels are damaged, platelets rapidly aggregate at the injury site, forming a platelet plug to block the rupture and releasing various factors related to blood clotting. This initiates a clotting cascade, promoting blood coagulation.

According to Balleisen et al. (1975) , Type III collagen affects the aggregation of human platelets. Subsequent studies have confirmed this finding. It is now known that platelets interact with Type III collagen through specific glycoproteins and non-integrin receptors. In 2008, Jarvis et al. used 57 synthetic peptides to test their interaction with human and mouse platelets. These peptides, derived from the COL3A1 sequence, can form a triple-helix structure. The study found that peptides containing three hydroxyproline residues bind to glycoprotein VI in platelets.

Inflamm-ation

Inflammation is a critical stage in normal wound healing. It is essential to resolve inflammation promptly during wound repair. Inflammation resolution is a dynamic process driven by a mix of pro-inflammatory and anti-inflammatory responses. Research using stable collagen matrices has shown that collagen induces a powerful but acute inflammatory response, which is transient and resolves quickly, promoting wound healing. Additionally, collagen has been demonstrated to play a key role in anti-inflammatory and pro-angiogenic macrophage phenotypes through microRNA signaling.

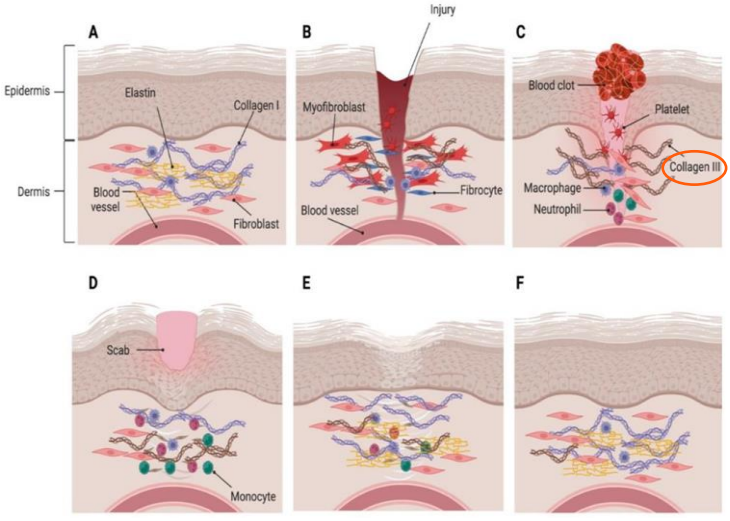
Prolifer-ation

After the inflammation response subsides and the epithelial layer thickens, fibroblasts inside the wound begin to proliferate. These cells are responsible for synthesizing and secreting collagen and constructing the extracellular matrix. As healing progresses, collagen fibers gradually become the primary structural component of the wound. The fiber bundles thicken, forming dense collagenous tissue, or scar tissue. This scar tissue helps securely reconnect the disrupted tissue. Since collagen is the primary protein providing tissue tensile strength, collagen metabolism plays a crucial role in wound healing.

Remod-eling

Once initial wound repair is completed, the internal structure must be adjusted to restore its physiological state. The new epithelium matures, and deep epithelial cells dissolve. The newly formed blood vessel network reduces and transforms into a more organized microvascular system. Collagen fibers become thicker, longer, and more organized, increasing tensile strength. Collagen maintains a high turnover rate under the action of collagenases, preserving tensile strength.

Fig. Different Stages of Injury and Subsequent Wound Healing Process



Source: Singh D, Rai V, Agrawal DK. Regulation of Collagen I and Collagen III in Tissue Injury and Regeneration. Cardiol Cardiovasc Med. 2023;7(1):5-16. doi: 10.26502/fccm.92920302. Epub 2023 Jan 20. PMID: 36776717; PMCID: PMC9912297.

Note: Different stages of injury and wound healing. A. Normal skin with epidermis and dermis. B. Blood flow to the injury site. C. Formation of a blood clot to prevent bleeding after injury. Macrophages facilitate tissue remodeling, with Type III collagen levels beginning to rise. D. Wound contraction and scab formation. E. Tissue maturation leads to scar tissue formation, with Type III collagen being replaced by Type I collagen during ECM remodeling. F. Restoration of normal skin after wound healing.

Sources: Public information, literature search, and Frost & Sullivan analysis.

2.2 Functions of Type III Collagen

Recent research has revealed that Type III collagen plays a crucial role in maintaining tumor cells in a dormant state within the tumor microenvironment, thereby inhibiting tumor proliferation.

2.2.5 Type III Collagen and Tumors

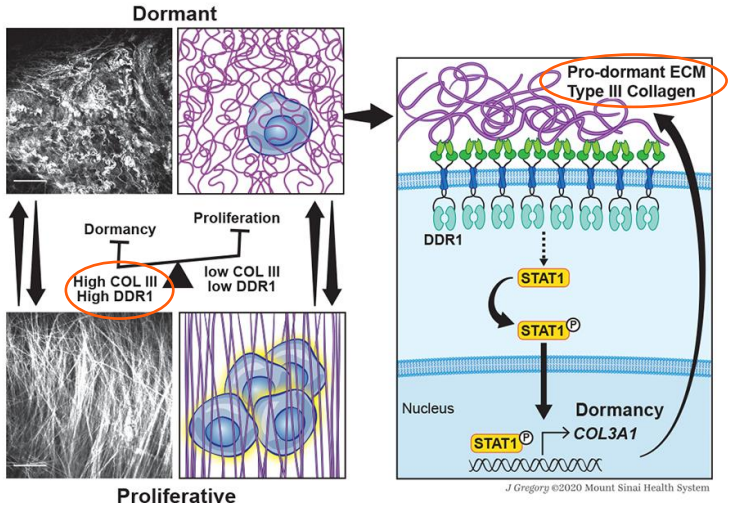
After the resection of a primary tumor, if residual tumor cells are not completely eradicated despite postoperative adjuvant therapy, these cells may migrate to distant organs through the bloodstream and establish themselves there. They subsequently enter a dormant state. After several years of latency, these dormant cells can be activated under specific conditions, rapidly re-entering the proliferation cycle and forming detectable metastases. Dormant tumor cells are in the G0/G1 phase, meaning they neither proliferate nor undergo apoptosis, making traditional radiotherapy and chemotherapy ineffective. This significantly increases the challenge of achieving a complete cure.

In 2021, an article published in Nature Cancer explored how disseminated tumor cells sense and remodel the extracellular matrix (ECM) to maintain dormancy. Through extracellular matrix proteomics analysis, researchers discovered that dormant cancer cells are situated in a microenvironment rich in Type III collagen. This collagen, produced by tumor cells, is vital for maintaining tumor dormancy. Disruption of this Type III collagen network leads to tumor cell reactivation via the DDR1-mediated STAT1 signaling pathway.

Researchers used second harmonic generation multiphoton microscopy to observe the transition of tumor cells from a dormant to an activated state. They noted changes in the structure and content of Type III collagen. Analysis of clinical samples showed that, compared to patients with positive lymph node metastases, those with negative lymph nodes had higher levels of Type III collagen in their tumors.

This study highlights the significant impact of the tumor microenvironment on tumor cell dormancy and demonstrates the critical role of Type III collagen in this process. Adding Type III collagen to the tumor microenvironment can promote and maintain tumor cells in a dormant state, thereby inhibiting tumor proliferation.

Fig. Dynamic Changes in ECM Structure Between Dormant and Proliferative Tumors



Source: Di Martino JS, Nobre AR, Mondal C, Taha I, Farias EF, Fertig EJ, Naba A, Aguirre-Ghiso JA, Bravo-Cordero JJ. A tumor-derived type III collagen-rich ECM niche regulates tumor cell dormancy. Nat Cancer. 2022 Jan;3(1):90-107. doi: 10.1038/s43018-021-00291-9. Epub 2021 Dec 13. PMID: 35121989; PMCID: PMC8818089.

Note: The ECM surrounding dormant cells is characterized by a wavy collagen matrix. Upon activation of dormant cells, this matrix reorganizes into a highly aligned structure. The ECM of dormant cells is rich in Type III collagen, contributing to the formation of a non-linear collagen ECM structure around these cells. Binding of DDR1 to Type III collagen activates the STAT1 signaling pathway, which triggers the reactivation of dormant cells and increases COL3A1 expression in disseminated tumor cells (DTCs), thus establishing a pro-dormancy ECM niche.

Sources: Public information, literature search, and Frost & Sullivan analysis.

2.3 Type III Collagen and Signal Transduction

The metabolism of Type III collagen involves a complex network of interrelated signal transduction pathways that collectively mediate the collagen metabolic process.

2.3.1 Type III Collagen and Signal Transduction

Protein metabolism ultimately begins with gene transcription. Generally, the transcriptional activity of genes is regulated by various cis- and trans-regulatory elements and signal transduction pathways composed of cytokines. Recently, fibrogenesis signal transduction pathways have garnered widespread interest. Studies have revealed that Type III collagen metabolism is regulated by signal transduction pathways such as NF-kB, TGF-β/Smads, PI3K/Akt, ERK, and Wnt. Research has shown that these pathways are interconnected and mutually influential, collaboratively mediating the collagen metabolic process.

NF-kB

The activation mechanism of the NF-kB signal transduction pathway involves the phosphorylation and ubiquitination of IkBα, leading to its degradation. This process releases NF-kB dimers, allowing NF-kB to enter the nucleus and initiate gene transcription. Studies have demonstrated that in the mRNA expression of Type III collagen, cathepsin B can chronically regulate the NF-kB signal transduction pathway. Cathepsin B directly degrades IkBα, causing chronic activation of the NF-kB signal transduction pathway, which in turn inhibits the mRNA expression of Type III collagen.

TGF-β/Smads

The TGF-β/Smads signal transduction pathway is a classic regulator of fibrogenesis, primarily involved in the deposition of extracellular matrix collagen. TGF-β is a multifunctional cytokine that regulates the growth, differentiation, and function of various cell types. Its primary role in mesenchymal cells is to stimulate ECM deposition. TGF-β induces the differentiation of myofibroblasts, increasing the expression of Type III collagen and leading to collagen deposition in the ECM. Activation of TGF-β begins with the binding of the ligand dimer to two type II TGF-β receptors (TGF-βR II), which then recruits and phosphorylates two type I receptors (TGF-βR I), forming a heteromeric complex with serine-threonine kinase activity. This complex activates downstream signal transduction pathways. TGF-βR II is essential for ligand specificity; without TGF-βR II, TGF-βR I cannot bind TGF-β alone, nor can TGF-βR II transmit signals alone. Once activated, TGF-βR I exhibits increased kinase activity toward intracellular Smad transcription factors. Activated TGF-βR I recruits and phosphorylates Smad2 and Smad3, which then associate with Smad4 to form a translocating complex that enters the nucleus. In the nucleus, this complex binds to target genes through other transcription factors (e.g., Sp1) and cooperates with CBP/p300 to promote the mRNA expression of Type III collagen.

ERK

The ERK pathway consists of ERK1 and ERK2, with the Ras/Raf/MEK/ERK pathway being the primary route. Studies have indicated that the ERK pathway is related to collagen metabolism. The serine protease inhibitor SERPINE2 suppresses MMP-13 activity by inhibiting the ERK1/2 pathway and downstream NF-kB transcription factors. For instance, inhibition of the ERK1/2 pathway leads to a significant decrease in the gene expression of Type III collagen. Additionally, inhibition of the ERK1/2 pathway in fibroblasts results in a marked reduction in their ability to secrete Type III collagen.

Wnt

The Wnt signal transduction pathway, which includes Wnt/β-catenin, Wnt/Ca2+, and Wnt/PCP routes, is associated with cell differentiation, growth, and apoptosis. The Wnt/β-catenin pathway is the most well-characterized and is involved in fibrosis processes in the liver, lung, and kidney. Studies have shown that transfecting β-catenin SiRNA into hepatic stellate cells results in a significant decrease in Type III collagen synthesis. Application of the Wnt pathway antagonist DKK-1 also leads to reduced levels of Type III collagen. MMP-13, a downstream molecule of the Wnt/β-catenin signal transduction pathway, can degrade Type III collagen. Further research has found that overexpression of β-catenin increases the expression of MMP-9 and MMP-13 genes in chondrocytes. Inhibitors targeting the key protein LRP5 in the Wnt/β-catenin pathway significantly reduce the mRNA expression of MMP-13 in chondrocytes, thereby decreasing the degradation of Type III collagen.

Sources: Public information, literature search, and Frost & Sullivan analysis.

Chapter 3

Recombinant Type III Triple-Helix Collagen

03

3.1 Introduction to Recombinant Type III Collagen

Animal-derived collagen processes are mature but involve safety risks. Recombinant Type III collagen offers greater safety and stability, though the technology faces high entry barriers.

3.1.1 Differences Between Recombinant Collagen and Animal-Derived Collagen

Source

Animal-Derived Collagen

- **Pigs and Cows:** The primary sources of animal-derived collagen are pigs and cows, from which types I, II, and III collagen can be extracted. The extraction technology for animal-derived collagen is relatively mature and widely used. Collagen is mainly extracted from four parts: tendon tissue, skin tissue, small intestine tissue, and cartilage tissue, each yielding different types of collagen.
- **Fish:** Collagen is extracted from fish scales, skin, and bones, which have high collagen content, primarily type I collagen. Most fish-derived collagen comes from wild tilapia and deep-sea cod.

Recombinant Collagen

- Recombinant Collagen: This method involves isolating gene fragments from human collagen, selecting the high-activity sequences for optimization and recombination, and using microbial fermentation to produce stable recombinant collagen.

Production Process

Animal-Derived Collagen

- **Process:** Animal-specific tissue → De-fatting → Slicing → Washing → Sterilization → Grinding → Reaction → Filtration → Purification → Obtain collagen solution. Key challenges in extracting animal-derived collagen include sterilization, virus inactivation, removal of immunogens, purification, and scaling up production.
- Acid Extraction: Collagen is dissolved in an acidic medium, followed by processes like salting out and dialysis to obtain acid-soluble collagen. This method preserves the triple-helix structure and maintains the biological properties of the collagen. It is cost-effective and offers high value. However, it has lower efficiency and retains terminal peptides, which increases the risk of immunogenicity.
- Enzymatic Extraction: This method uses proteolytic enzymes to remove non-helical terminal peptides from collagen. Common enzymes include pepsin, papain, and trypsin. It preserves the triple-helix structure, ensuring high safety and purity, stable physicochemical properties, and relatively higher extraction efficiency. Using a combination of enzymes can produce multifunctional collagen.

Recombinant Collagen

- **Process:** Obtain target genes → Construct and amplify plasmids → Cultivate bacterial strains → Seed culture → Fermentation production → Separation → Homogenization → Separation → Purification → Obtain recombinant collagen.
- In the preparation of recombinant Type III collagen, researchers use various technologies and strategies to optimize gene sequences, making them compatible with the host expression system. Gene cloning and transfection are employed to introduce the genes into the host, where the protein is synthesized using the host's biosynthetic machinery. Purification methods, such as affinity chromatography and gel electrophoresis, are used to improve purity. Key cost factors in production include the selection of upstream protein sequences, expression systems, cell culture, and cell transfection.

Fig. Comparison of Animal-Derived Collagen and Recombinant Collagen

	Application Range	Physicochemical Properties	pH Value and Viscosity	Advantages	Limitations
Animal-Derived Collagen	Food, skincare products, medical devices, etc.	Complete triple-helix structure and biological activity	Slightly acidic, high viscosity	• Relatively mature extraction technology • Lower threshold and cost	• Risk of animal-borne disease infections; • Allogeneic collagen may cause immune rejection or allergic reactions; • Production capacity limitations; • Difficulty in controlling purity, limited applications
Recombinant Collagen	Food, skincare products, medical devices, and special applications in tissue engineering and regenerative medicine.	Higher safety and biocompatibility; can also produce collagen with a triple-helix structure	Soluble in neutral liquids, low viscosity	• Product sequence highly consistent with human gene sequence; good tissue compatibility; • High safety, strong processability, stable quality, high water solubility, and emulsification	• High technical threshold; • Currently higher cost; • Technical challenges in large-scale production and long-term storage

Sources: Public information, literature search, and Frost & Sullivan analysis.

3.1 Introduction to Recombinant Type III Collagen

Recombinant Type III collagen can be distinguished based on gene sequence, protein structure, and formulation type. The preparation process, application scenarios, and advantages vary accordingly.

3.1.2 Classification of Recombinant Type III Collagen

According to the "Guidelines for Naming Recombinant Collagen Biomaterials" issued by China's National Medical Products Administration, recombinant Type III collagen refers to "recombinant collagen based on the COL3A1 gene or part of its gene sequence." It can be categorized based on the following aspects:

By Collagen Composition and Structure

Recombinant Human Collagen

"Recombinant human collagen" refers to collagen produced using DNA recombination technology, where the full-length amino acid sequence is encoded by specific human collagen genes and retains a triple-helix structure. If the recombinant collagen lacks the triple-helix structure, despite being derived from the full-length human collagen gene sequence, it is not classified as "recombinant human collagen." This collagen, synthesized directly from human collagen DNA sequences, is identical to naturally occurring human collagen in amino acid composition, offering high biocompatibility and safety. However, production costs remain high.

Recombinant humanized Collagen

"Recombinant humanized collagen" is further divided into Type A and Type B, depending on the presence of non-human collagen sequences. Type A includes materials that do not contain non-human collagen amino acid sequences, which can be either full-length human collagen gene sequences, partial amino acid fragments, or combinations of functional human collagen segments. Type B includes non-human amino acid sequences, such as linker or tag sequences, added to functional human collagen fragments. This type of collagen is also derived from human cells, but specific gene fragments are assembled to create new proteins with distinct structures and functions. This retains the physiological activity of collagen while reducing molecular weight.

Recombinant Collagen Variants

"Recombinant Collagen Variants" refers to materials where the gene or amino acid sequence shows low homology to human collagen. This classification solely reflects the levels of homology. It does not suggest that medical devices made with Recombinant Collagen Variants are inferior in safety, efficacy, or quality compared to those made from recombinant human or humanized collagen. This type of collagen is produced by combining human collagen genes with collagen genes from other species. Scientists integrate active fragments from human and animal collagen genes, resulting in highly functional collagen. However, due to the involvement of animal-derived gene sequences, there may be potential risks of immunogenicity or allergic reactions.

By Collagen Formulation Type

Fig. Classification of Recombinant Collagen (by Formulation Type)

	Main Application Areas	Advantages
Solution	Wound healing dressings, injectable materials	Easy to use, no need for reconstitution
Lyophilized Powder	Wound healing dressings, injectable materials	Freeze-drying process for easy storage and transportation
Gel	Wound healing dressings	Keeps the wound moist, promoting better healing
Sponge	Wound dressings	Absorbs large amounts of exudate, prevents friction damage and contamination
Fiber	Injectable materials after reconstitution	Ultra-low temperature freeze-drying process ensures more stable collagen activity

Sources: Public information, government websites, literature search, and Frost & Sullivan analysis.

3.2 Industry Standards for Recombinant Collagen

Policies and standards for recombinant collagen are crucial for market regulation, production guidance, and ensuring safety and efficacy, offering quality control guidelines and promoting development in biomaterials and medical devices.

3.2.1 Policies and Industry Standards for Recombinant Collagen

China has established a series of industry standards and policy regulations for recombinant collagen products. These documents detail the quality control requirements, testing indicators, and methods for recombinant collagen. For example, YY/T 1849-2022 "Recombinant Collagen" is a key industry standard published in January 2022 and officially implemented in August 2022. Additionally, YY/T 1888-2023 "Recombinant humanized collagen protein" is a medical device industry standard released in January 2023 and implemented in July 2023. These two standards cover the quality control requirements, testing indicators, and methods for recombinant collagen, as well as the definitions, classifications, inspection, labeling, usage, and safety of recombinant human-like collagen. These industry standards apply to the quality control of recombinant collagen and recombinant human-like collagen used as raw materials for medical devices. The formulation and implementation of these standards are crucial for regulating market order, guiding enterprise production activities, and ensuring the safety and efficacy of end products. They not only provide clear guidance for the quality control of recombinant collagen in medical device raw materials but also encourage innovation in new biomaterials and promote high-quality development in the medical device industry.

March 2021 **NMPA** **"Guidelines for Naming Recombinant Collagen Biomaterials"**	• Recombinant collagen biomaterials are named using a structure of "feature word + core word (A+B)", such as "Recombinant Type III Human-Like Collagen Solution". Core words and feature words should be selected based on their true attributes and characteristics, with priority given to terms from the terminology table.
April 2021 **NMPA** **"the Principles for the Classification Defining of Recombinant Collagen Products"**	• Recombinant collagen products should be classified no lower than Class II. When used as non-implantable medical devices, they should be managed as Class III medical devices. If used as medical dressings, and if the product is partially or fully absorbed by the human body or used for chronic wounds, it should be managed as Class III medical device; otherwise, it should be managed as Class II.
October 2022 **Ministry of Commerce Research Institute** **"White Paper of Development of High-Quality Collagen Industry in China"**	• The focus is on pursuing high-quality development by enhancing cooperation among industry, academia, research, and government. It emphasizes improving production processes, adding value to products, extending the product chain, and upgrading products. It also highlights the importance of strengthening oversight from the source, focusing on standard development and standardized application, and encouraging companies to retain high-end products domestically to drive domestic consumption upgrades.
December 2022 **NMPA Medical Device Evaluation Center** **"Review Key Points of Recombinant Humanized Collagen Injection Materials for Plastic Surgery (Draft)"**	• This document confirms that the management category for recombinant human-like collagen injection materials for plastic surgery is Class III and standardizes its management category, application materials, technical requirements, etc.
January 2023 **NMPA** **"Recombinant humanized collagen protein" Industry Standard**	• This standard regulates the quality control, technical requirements, testing methods, stability, biological evaluation, and packaging, transportation, and storage of recombinant human-like collagen. It applies to quality control of recombinant human-like collagen used as raw materials for medical devices that do not contain non-human collagen amino acid sequences.
April 2023 **China Anti-Aging Promotion Association** **"Quality requirements and efficacy assessment for recombinant collagen"**	• This document specifies the terminology and definitions, quality requirements, testing methods, efficacy evaluation, and inspection rules for recombinant collagen. It applies to the quality requirements and efficacy evaluation of recombinant collagen used as a cosmetic ingredient.
April 2023 **China Anti-Aging Promotion Association** **"Group Standard for Recombinant Collagen Raw Materials for Cosmetics"**	• This standard outlines the basic requirements for recombinant collagen used in cosmetics, including basic information, testing items, requirements and methods, toxicology, stability, packaging, transportation, and storage. It applies to the quality control of recombinant collagen used in cosmetics.
May 2023 **NMPA Medical Device Evaluation Center** **"Guidelines for the Registration Review of Recombinant Collagen Wound Dressings"**	• These guidelines apply to recombinant collagen wound dressings managed as Class II medical devices, containing recombinant collagen components, and used for non-chronic wounds and surrounding skin care. Products are typically in forms such as gels, liquids, patches, or creams, including single-use and multiple-use products, available in sterile or non-sterile forms.
May 2023 **NMPA Medical Device Evaluation Center** **"Guideline on Recombinant Humanized Collagen Raw Materials"**	• This document guides the evaluation principles and application materials of all recombinant human-like collagen raw materials used in implantable medical devices. It also offers reference points for evaluating other medical device applications of recombinant collagen materials.

Sources: Public information, government websites, literature search, and Frost & Sullivan analysis.

3.3 Preparation Process and Key Technologies for Recombinant Type III Collagen

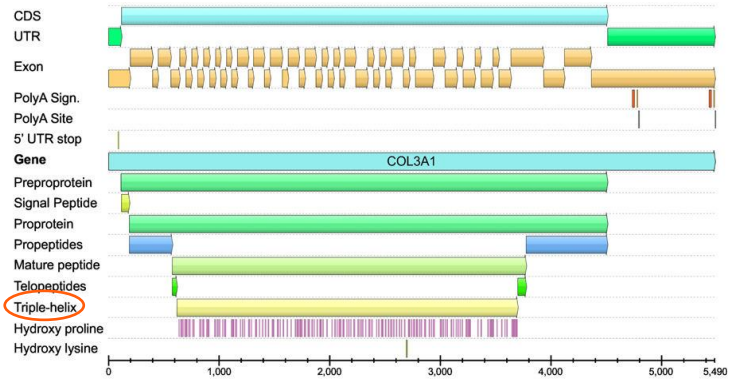
The formation of the Type III collagen triple-helix structure depends on post-translational modifications. High-level expression of the triple-helix region can be achieved through sequence optimization and selection, along with choosing or modifying the expression system.

3.3.1 Key Technologies for the Preparation of Recombinant Type III Collagen—Sequence Design and Optimization

The COL3A1 gene, which encodes the α1 chain of Type III collagen, is located on the long arm of chromosome 2 in the human genome. This gene is approximately 38 kb in length, contains 51 exons, and is numbered 152, consistent with the exon numbering of other fibrillar collagen genes.

In the sequence design and optimization process for recombinant Type III collagen, key considerations include: (1) incorporating crucial sites for cell adhesion and signaling, such as GER or GEK integrin recognition sites; (2) the molecular weight of the recombinant collagen; (3) selecting sequences that enhance expression levels and stability for more efficient production; and (4) choosing sequences that form a stable triple-helix structure to better achieve its biological function.

Fig. Human COL3A1 Transcript Structure



Source: Kuivaniemi H, Tromp G. Type III collagen (COL3A1): Gene and protein structure, tissue distribution, and associated diseases. Gene. 2019 Jul 30;707:151-171. doi: 10.1016/j.gene.2019.05.003. Epub 2019 May 7. PMID: 31075413; PMCID: PMC6579750.

3.3.2 Key Technology in Recombinant Type III Collagen Preparation—Expression System Selection and Optimization

Recombinant collagen expression systems include prokaryotic, yeast, plant, insect, and mammalian cell systems. The characteristics of collagen make it an important structural and cell signaling molecule, primarily due to: (1) the heat-stable triple-helix structure composed of specific α-chains; (2) precise post-translational modifications of α-chains; (3) correct processing of the propeptide segments; (4) the ability to form supramolecular complexes. These characteristics are not only closely related to the assembly process of collagen itself but are also significantly influenced by various collagen-modifying enzymes present in the recombinant collagen expression system. Therefore, to successfully prepare and produce recombinant collagen with a triple-helix structure, selecting and/or modifying an expression system that contains necessary collagen-modifying enzymes is crucial. Currently, Escherichia coli and yeast are the main expression systems, but these systems lack the collagen post-translational modification capabilities found in animal cells, so corresponding recombinant enzymes need to be introduced. Meanwhile, although plant, baculovirus, and mammalian cell expression systems produce collagen at much lower yields compared to E. coli and yeast systems, they can generate collagen with complete triple-helix structures and good thermal stability.

Fig. Recombinant Type III Collagen Expression Systems

	Advantages	Limitations
E. coli expression system	Clear genetic background, low fermentation cost, short production cycle, high efficiency	Produced collagen lacks hydroxylation
Yeast expression system	Capable of modifying secreted proteins, produced proteins are free of pathogens, viral inclusions, or pyrogens, high safety, low fermentation cost, high yield	Difficult to produce heterologous collagen
Plant expression system	Scalable, short production cycle, low cost, high safety	Low yield, insufficient production capacity
Insect expression system	Low background interference, strong post-translational modification capabilities	Long expression cycle, low yield
Mammalian cell expression system	Stable expression, stable yield	Long expression cycle, high cost, high requirements for the culture system, susceptible to viral infections

Sources: Public information, literature search, and Frost & Sullivan analysis.

3.3 Preparation Process and Key Technologies for Recombinant Type III Collagen

Successful expression of recombinant Type III collagen requires precise control of cell lysis and purification. Its physical and chemical properties, structural characteristics, thermal stability, and biological functions must also be evaluated according to national standards.

3.3.3 Key Technologies in Recombinant Type III Collagen Preparation—Expression, Purification, and Testing

The successful expression of recombinant Type III collagen requires further lysis and purification. The lysis process involves physical methods, such as ultrasonication or high-pressure homogenization, or chemical methods, such as detergents and solubilizers, to break down host cell walls and membranes, releasing the protein. Initial purification steps include precipitation, centrifugation, and filtration to remove most impurities, such as unbroken cells and cell debris. Further purification typically involves chromatographic techniques, including ion exchange, affinity chromatography, gel filtration, and liquid chromatography, to isolate the target protein based on properties such as charge, hydrophilicity, size, and binding affinity.

Fig. Partial Evaluation Standards for Recombinant Collagen in China

Identification:
- Amino acid sequence and coverage; peptide mapping; molecular weight; isoelectric point; thiol groups and disulfide bonds; extinction coefficient (or molar absorptivity); electrophoretic pattern; liquid chromatography profile.

Structural Characterization:
- Proline hydroxylation
- Higher-order structure analysis
  - triple-helix structure analysis:
    - Qualitative: The circular dichroism (CD) spectrum of collagen shows a negative peak around 195 nm and a positive peak around 221 nm. If the positive peak at 221 nm is absent, it indicates the lack of a triple-helix structure.
    - Quantitative: A series of collagen standards mixed in different ratios with fully denatured collagen standards can be used as external references to fit the intensity of the positive peak near 221 nm.
  - Fiber quality/porous network structure characterization

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), or atomic force microscopy (AFM).

Purity:
- Electrophoresis

Content Determination:
- High-performance liquid chromatography (HPLC), Kjeldahl nitrogen method, characteristic peptide method.

Impurities, Contaminants, and Additives:
- Bacterial endotoxins, microbial limits, sterility.

Thermal Stability:
- Differential scanning calorimetry (DSC); observations after exposure to a water bath.

Other Physical and Chemical Indicators:
- Appearance, visible particulates, solubility, moisture content, ash residue, pH, osmolarity, dynamic viscosity (gel), dosage and variation, degradation characteristics, and products.

Biological Function Evaluation:
- Cell-collagen interactions.

Sources: Public information, government websites, literature search, and Frost & Sullivan analysis.

3.4 Recombinant Type III Triple-Helix Collagen

Type III collagen consists of three peptide chains forming a stable triple helix. This structure provides collagen with strength and stability and directly influences its function in the body.

3.4.1 Characteristics and Advantages of the Recombinant Collagen Triple-Helix Structure

The Triple-Helix structure of collagen, composed of three polypeptide chains, has the following characteristics:

1	**Basic Unit**	• Tropocollagen is the basic unit of collagen, consisting of three polypeptide chains.
2	**Gene Sequence**	• Each polypeptide chain's primary structure has a repeating (Gly-X-Y)n sequence, where X and Y are typically proline and hydroxyproline.
3	**Helical Structure**	• Each collagen chain adopts a left-handed helical conformation, and the three left-handed helices twist together to form a right-handed superhelix.
4	**Active Collagen**	• Collagen with a complete triple-helix structure is called active collagen, which possesses biological activity and can promote tissue growth and repair. It is widely used in cosmetics, medicine, and tissue engineering.

There is a Close Relationship Between the Triple-Helix Structure of Collagen and Its Physiological Activity

The Triple-helix Structure Plays a Key Role in the Mechanical Strength of Recombinant Collagen

The mechanical properties of recombinant collagen are crucial for its use in aesthetic fillers and tissue engineering materials. These properties are determined by its chemical composition, degree of cross-linking, and the presence of a triple-helix structure. The natural triple-helix structure is essential for collagen's high mechanical strength. Stress-strain tests have shown that natural collagen exhibits both elastic and viscoelastic properties, while denatured collagen, which lacks the triple-helix, shows limited elasticity and only half the tensile strength of natural collagen. This highlights that the triple-helix structure is key to maintaining collagen's mechanical integrity.

The Triple-helix Structure Enhances Interaction Between Recombinant Collagen and Other Components

As a major component of the skin's extracellular matrix, collagen not only serves as an anchor and structural support for cells but also creates a conducive microenvironment for cellular growth. It participates in cell migration, differentiation, and proliferation, promoting the growth of various cell types. The natural collagen structure, especially its well-developed tertiary structure, ensures its ability to interact with other extracellular matrix proteins, such as elastin and fibronectin, forming a specific 3D network that supports cell function. Moreover, the triple-helix structure ensures that active binding sites for proteins like integrins are exposed, facilitating effective signal transduction.

The Triple-helix Structure Slows Down the Degradation Rate of Recombinant Collagen

Collagen can only be degraded by collagenase, which cleaves its peptide chains and disrupts the triple-helix structure, initiating hydrolysis. These hydrolyzed fragments can then spontaneously denature at body temperature and are further broken down by other enzymes into oligopeptides or amino acids, which are either utilized by the body or excreted. Single-stranded recombinant collagen, lacking the triple-helix structure, degrades rapidly in the body, leading to a shorter duration of action. In contrast, recombinant collagen with a triple-helix structure is more resistant to degradation and can persist in the body for extended periods. This characteristic is crucial for its effectiveness in cosmetic and aesthetic applications.

Sources: Public information, government websites, literature search, and Frost & Sullivan analysis.

3.4 Recombinant Type III Triple-Helix Collagen

Everon Healthcare's high-level biological experiment platform supports the development of high-quality recombinant type III collagen.

3.4.2 Recombinant Type III Triple-Helix Collagen Business Case – Everon Healthcare

■ **Everon Healthcare High-Standard Biological Laboratory Platform**

AI-Assisted Protein Design Platform

Everon Healthcare utilizes AI algorithms to predict, design, and construct multi-copy clones that incorporate the molecular characteristics of natural human collagen genes. The expressed proteins retain the superior qualities of natural human collagen, enabling high-level expression of recombinant humanized collagen both intracellularly and extracellularly.

The multi-copy clones developed by Everon Healthcare are highly scalable and, under high-density fermentation conditions, can produce recombinant collagen in large quantities. The extracellular secretion of the collagen simplifies product separation and purification, reducing production costs. Moreover, the recombinant humanized collagen, designed and expressed using AI algorithm, has a stable composition and biological activity. It exhibits high safety, being less likely to trigger immune rejection when applied to the human body. Transmission electron microscopy (TEM) confirms that Everon's recombinant collagen has a natural triple-helix structure, with biological activity and functionality comparable to natural collagen extracted from animal sources.

Advantages:

- ✓ Increase production throughput.
- ✓ Significantly improve process yield and success rate.
- ✓ Shorten production cycles.

High-Throughput Structural Biology Platform

Leveraging extensive expertise in molecular biology, structural biology, and protein biochemistry, Everon Healthcare has developed an advanced high-throughput platform for protein cloning, expression, and purification. Built on mature, well-established high-throughput techniques from Oxford University, this platform optimizes workflows, improves experimental efficiency, increases speed and throughput, shortens project timelines, and reduces costs.

Advantages:

- ✓ Transit traditional laboratories towards high-throughput, scaled, and automated processes.
- ✓ Provide efficient support and validation for front-end sequence computation and design.

Host Strain Modification Platform

By integrating the DBTL (Design-Build-Test-Learn) cycle with traditional biology, and applying principles of systems design and engineering, Everon Healthcare has developed and screened high-quality, high-yield, and stable engineered strains. These include strains such as Escherichia coli, Lactococcus lactis, Pichia pastoris, and Saccharomyces cerevisiae.

Advantages:

- ✓ Accurately identify and locate target genes, enabling personalized modifications of base strains.
- ✓ Greatly enhance experimental efficiency and shorten development cycles.

Protein Raw Material Pairing, Screening, and Validation Platform

International Standard Protein Purification and Production Platform Capabilities »

- Immunochromatography Screening
- ELISA
- Molecular Interaction Measurement
- Functional Biochemical Experiments
- Thermal Stability Testing

Advantages:

- ✓ Develop diverse and complex proteins.
- ✓ Validate using proprietary technology platforms.
- ✓ Conduct high-throughput experimental screening and iterative optimization.
- ✓ Deeply customize applications.

Fig. Everon Healthcare High-Standard Biological Laboratory Platform

High-Throughput Protein Purification Platform | 200L Fermentation Tank | AKTA pure™ 25 | AKTA flux™ 6

Source: Public information, literature search, and Frost & Sullivan analysis.

3.4 Recombinant Type III Triple-Helix Collagen

Under common usage conditions, recombinant Type III collagen does not cause harm or induce hazardous reactions to humans or the environment and has no cytotoxicity.

Physicochemical Properties of Recombinant Type III Triple-Helix Collagen from Everon Healthcare

Under normal usage conditions, recombinant Type III collagen does not cause harm or induce hazardous reactions in humans or the environment, and exhibits no cytotoxicity. Regarding the safety of recombinant Type III collagen, based on Everon Healthcare's safety data sheet for recombinant Type III collagen, the summary is as follows.

Fig. Physicochemical Properties of Recombinant Type III Collagen from Everon Healthcare

Physical and Chemical Properties	• Recombinant Type III collagen is a white or off-white lyophilized powder or sponge-like solid with no smell. • It is readily soluble in water and dissolves in diluted inorganic acids, organic acids, and polar solvents. • Its flammability is not significant; it is difficult to ignite and does not spontaneously combust. • It does not undergo hazardous reactions, such as polymerization or self-decomposition, and does not produce flammable gases when in contact with water.
Health Hazards	• According to the hazard overview section of the safety data sheet, this substance is not classified as hazardous and does not pose health risks under normal conditions. • Inhalation: Under normal conditions, it does not generate dust. Even if inhaled, it does not cause significant irritation. • Ingestion: It is not notably toxic, but large amounts may cause gastrointestinal discomfort. • Eye Contact: It is not irritating; rinse with water if contact occurs. • Skin Contact: It is not irritating; wash with water and soap. • No special treatment is required after exposure, and there is no life-threatening danger.
Safety Response	• General washing measures should be taken after exposure; no special physical or chemical treatment is required. • No special protective equipment or emergency procedures are needed. • Avoid dust accumulation and inhalation; a mask can be worn during handling.
Stability & Reactivity	• Chemically stable under normal environmental conditions; does not undergo hazardous reactions. • Avoid acidic and alkaline conditions, as well as high temperatures. • Does not react hazardously with other substances (e.g., polymerization, decomposition). • Not a self-reactive or naturally decomposing substance.
Toxicological Information	• Acute Toxicity: Oral LD50 > 15,000 mg/kg (rat). • Irritation: No irritation to skin or eyes. • Sensitization: No sensitization reactions. • Chronic Toxicity: No clear evidence of chronic toxicity.
Ecological Impact	• Does not have adverse effects on the environment. • No special disposal methods are required.

Sources: Public information, government websites, literature search, and Frost & Sullivan analysis.

3.4 Recombinant Type III Triple-Helix Collagen

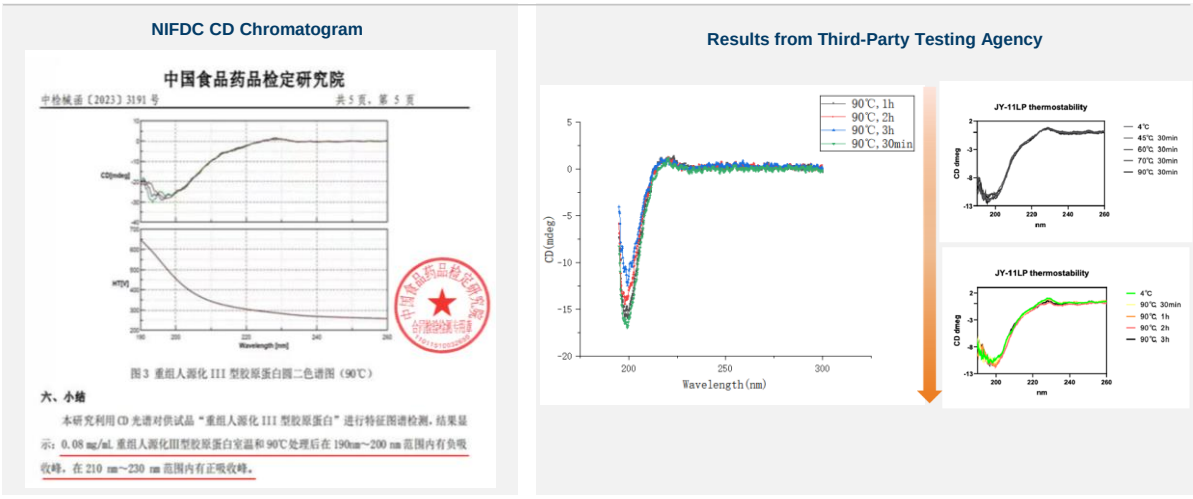
Everon's Exquisite Triple-Helix™ Recombinant Type III Collagen offers 90°C thermal reversibility, "360°" water molecule binding capability, self-assembling quaternary structure, and superior firming and anti-wrinkle effects.

■ Features and Advantages of Everon Healthcare's Recombinant Type III Triple Helical Collagen

□ 90℃ Thermal Reversibility

When collagen is used in cosmetics or medical devices, it must be able to withstand high temperatures or undergo sterilization. However, the triple-helix structure of collagen is often unstable at high temperatures. Thermal denaturation can significantly alter the properties of collagen, reducing its mechanical strength and enzymatic stability, which in turn diminishes its value for biomedical applications. Everon Healthcare's recombinant humanized collagen, after being treated at 90°C, shows a negative absorption peak at 190nm-200nm and a positive absorption peak at 210nm-230nm, confirming the high thermal stability of its triple-helix structure.

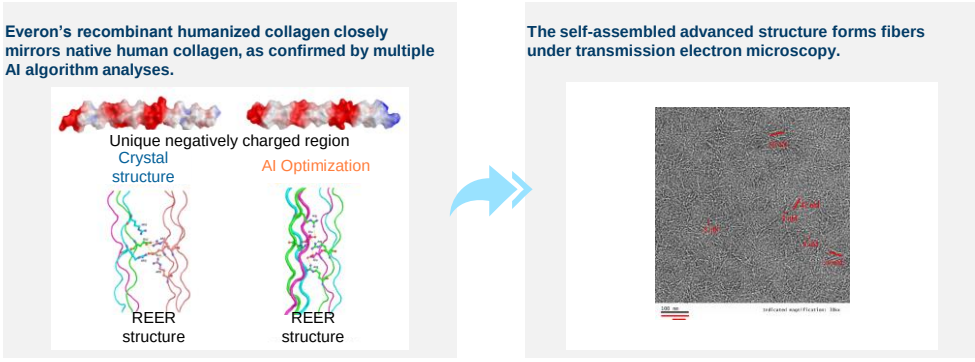
Fig. Everon's Recombinant Type III Triple-helix Collagen Testing Results



□ Self-assembled Quaternary Structure

In the body, collagen molecules self-assemble into orderly structures with specific functions. Self-assembly is a process where collagen molecules spontaneously organize into more complex structures through intermolecular interactions. This structure plays an important role in cell adhesion, proliferation, spreading, and migration. Through self-assembling, collagen can form highly ordered network structures, which show great potential in fields such as regenerative medicine, drug design, and tissue engineering. Transmission electron microscopy has confirmed that Everon Healthcare's recombinant Type III collagen retains its natural triple-helix structure and can further self-assemble into quaternary structures, exhibiting bioactivity and functionality equivalent to that of natural collagen extracted from animal sources.

Fig. Quaternary structure of Everon's recombinant Type III collagen confirmed by AI algorithms and transmission electron microscopy



Sources: Public information, government websites, literature search, and Frost & Sullivan analysis.

3.4 Recombinant Type III Triple-Helix Collagen

Everon's Exquisite Triple-Helix™ Recombinant Type III Collagen offers 90°C thermal reversibility, "360°" water molecule binding capability, self-assembling quaternary structure, and superior firming and anti-wrinkle effects.

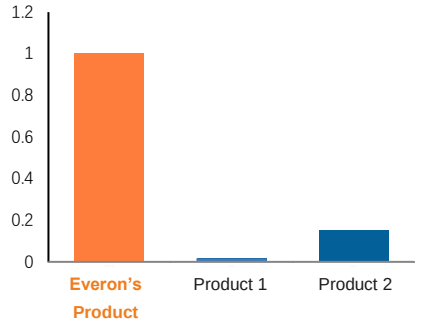
360° Water Molecule Binding Strength

The triple-helix structure is the foundation of collagen's biological function. This structure is formed by three α-chains tightly connected through hydrogen bonds, allowing it to bind with water molecules and undergo hydration, thereby creating a hydrated matrix with exceptional water-retention properties.

An increased level of hydroxylation enhances the hydrophilicity of collagen, further improving its water-retention performance.

Everon Healthcare's recombinant Type III collagen binds 763 water molecules for every 29 amino acids, showcasing superior water-retention capabilities.

Fig. Hydroxyproline Content Test of Everon's Recombinant Type III Collagen



Outstanding Repair and Anti-Aging Efficacy

Elastic proteins play a key role in maintaining skin elasticity but can be broken down by elastase in the body. Although elastase is widely distributed, the body normally maintains a dynamic balance with natural inhibitors that suppress elastase activity. However, aging, genetic defects, prolonged UV exposure, or inflammation can disrupt this balance, leading to the degradation of elastic proteins. Everon Healthcare's recombinant Type humanized III collagen demonstrates a high rate of elastase inhibition, effectively reducing the breakdown of elastic proteins in the skin and providing excellent firming and anti-aging benefits.

Additionally, Everon Healthcare's recombinant Type III collagen promotes cell proliferation, enhances fibroblast adhesion, and facilitates cell migration, resulting in noticeable wrinkle reduction and skin tightening.

Fig. Everon Healthcare's Recombinant Type III Collagen Elastase Inhibition Rate

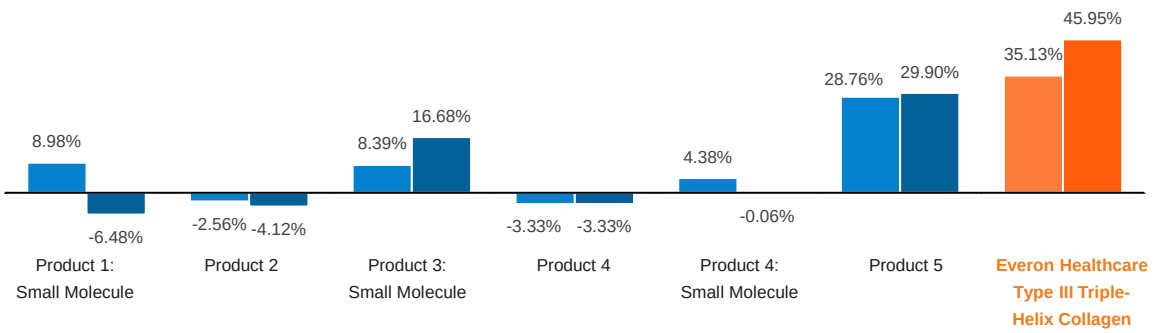


Fig. Everon's Recombinant Type III Collagen Anti-Wrinkle Clinical Trial

- **Materials:** Everon Healthcare's recombinant Type III triple-helix humanized collagen solution.
- **Methods:** Utilized T/ZHCA 006-2019 and T/TDCA 003-2021 cosmetic anti-wrinkle efficacy testing methods.
- **Results:**

Sources: Public information, government websites, literature search, and Frost & Sullivan analysis.

Chapter 4

Applications of Recombinant Type III Triple-Helix Collagen

04

4.1 Market Analysis of Recombinant Collagen Products

Collagen is widely used in aesthetic medicine, Efficacy-Driven Skincare, serious medical applications, and other consumer fields. The market for recombinant collagen, including Type III, is expanding rapidly due to its unique advantages and increasing market penetration.

4.1.1 Market Analysis of Recombinant Collagen and Recombinant Type III Collagen Products

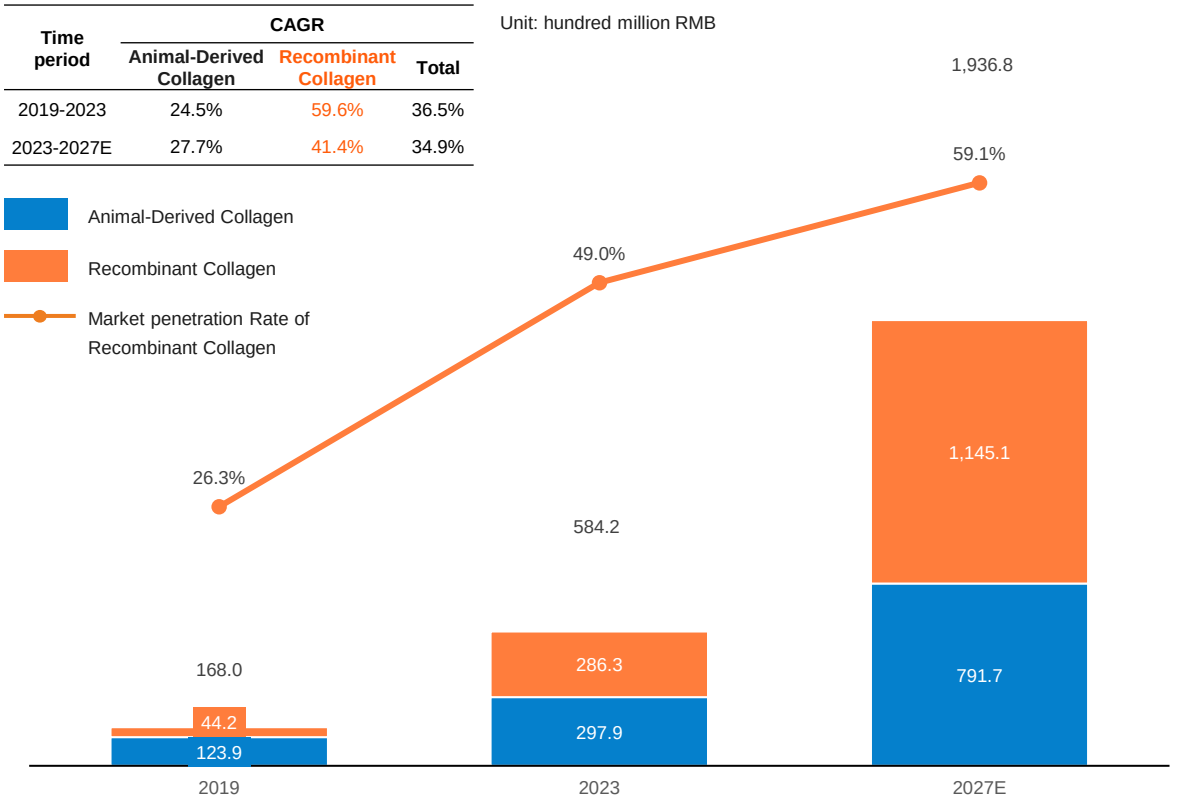
Recombinant Type III collagen is used in aesthetic medicine, efficacy-driven skincare, serious medical applications, and other consumer fields. According to the National Medical Products Administration (NMPA) website, as of August 31, 2024, around 170 recombinant Type III collagen-based medical devices have been approved in China, including scar gels, dressings, ointments, repair solutions, and lyophilized fibers. These products are used in areas such as aesthetic injections, wound repair, scar prevention, and gynecology.

In recent years, the recombinant collagen market in China has been booming, drawing strong interest from both investors and consumers. The approval of Class III medical devices made from recombinant Type III collagen has prompted numerous companies to enter this sector, leading to expanded production capacity and accelerated industrialization.

- According to Frost & Sullivan's analysis, from 2019 to 2023, the market size of China's collagen in the retail segment increased from 16.8 billion RMB to 58.4 billion RMB, with a CAGR of 36.5%. It is expected to reach 193.7 billion RMB by 2027.
- In 2023, the market size of recombinant collagen in the retail segment was 28.6 billion RMB, accounting for 49% of the overall collagen retail market size. By 2027, it is projected that the market size of recombinant collagen will reach 114.5 billion RMB, accounting for 59% of the overall collagen market size. Recombinant Type III collagen, composed of three identical peptide chains, has emerged as one of the fastest-developing types in the field of recombinant collagen research and application.

Fig. 2019–2027E Market Size of Collagen Products in China (Retail End)

Time period	CAGR		
	Animal-Derived Collagen	Recombinant Collagen	Total
2019-2023	24.5%	59.6%	36.5%
2023-2027E	27.7%	41.4%	34.9%



Sources: Public information, government websites,and Frost & Sullivan analysis.

4.2 Applications of Recombinant Type III Collagen — Serious Medical Applications

Due to its unique properties such as biocompatibility, biodegradability, promotion of cell proliferation, and hemostatic capabilities, recombinant Type III collagen can be applied in various serious medical fields.

4.2.1 Applications of Recombinant Type III Collagen in Serious Medical Fields

Recombinant Type III collagen has extensive prospects in serious medical applications due to its advantages such as low immunogenicity, high biocompatibility, and excellent hemostatic properties. Collagen-based formulations are primarily used to treat the following:

■ **Musculoskeletal System:**

Conditions such as hip or knee osteoarthritis, sprain-induced knee pain, cartilage damage, piriformis syndrome, arthritis or fusion of the ankle and hindfoot, lumbar fusion, myofascial pain syndrome, chronic pain, acute lumbar pain, partial rotator cuff tears, plantar fasciitis, calcific tendinitis, and pain.

■ **Urogenital System:**

Conditions like urinary incontinence, neurogenic bladder incontinence, lichen sclerosus, intrinsic sphincter deficiency, post-prostatectomy urinary incontinence, etc.

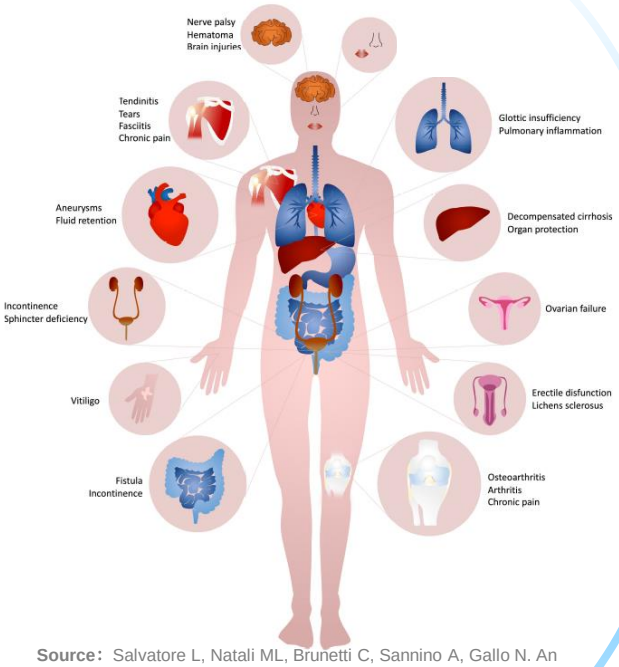
■ **Gastrointestinal System:**

Conditions such as glottic insufficiency, rectal fistula, fecal incontinence, etc.

■ **Other Applications**

Facial nerve rehabilitation post-facial paralysis, organ protection during thermal ablation, excessive inflammation related to COVID-19, ovarian function recovery in cases of premature ovarian failure, aneurysm closure, and blood volume expansion.

Fig. Main Clinical Applications of Collagen Products



Surgical Sutures
Absorbable and degradable by the body, eliminating the need for suture removal

Hemostatic Fibers
Adhesion to tissues and wound closure while stimulating the growth of fibroblasts

Plasma Substitute
Hydrolyzed collagen colloid solutions can expand blood volume

Hydrogels
Used for medical dressings, drug carriers, and tissue engineering scaffolds

Artificial Skin
Guides the skin through rapid scarless healing

Artificial Blood Vessels
Collagen-coated artificial vessels offer both biocompatibility and mechanical strength

Artificial Bones and Bone Repair
Provides excellent biocompatibility and osteoconductivity

Corneas
Provides patients with a clear and stable cornea without the need for immunosuppressive drugs

Source: Salvatore L, Natali ML, Brunetti C, Sannino A, Gallo N. An Update on the Clinical Efficacy and Safety of Collagen Injectables for Aesthetic and Regenerative Medicine Applications. Polymers (Basel) 2023 Feb 17;15(4):1020. doi: 10.3390/polym15041020.

Sources: Public information, literature search, and Frost & Sullivan analysis.

4.2 Applications of Recombinant Type III Collagen — Aesthetic Medicine

Recombinant Type III collagen can be used as a Class III medical device for dermal injections or as a Class II medical device to promote skin wound healing, with its efficacy supported by multiple studies.

4.2.2 Applications of Recombinant Type III Collagen in Aesthetic Medicine

Recombinant Type III collagen is widely used in aesthetic medicine for skin regeneration and repair, cosmetic enhancement, and anti-aging. It is effective in treating various skin injuries and conditions such as burns, wounds, and scars. It promotes the proliferation and differentiation of skin cells, accelerates skin regeneration and repair, and improves skin appearance and texture. Clinical trials both domestically and internationally have confirmed the value of recombinant Type III collagen in aesthetic medicine.

■ **Aesthetic Injectable Products (Class III Medical Devices)**

Before 2021, collagen injectables for aesthetic purposes in China were primarily derived from animal tissue. In 2021, China approved its first recombinant Type III humanized collagen freeze-dried fibers for aesthetic injections, sparking a surge in recombinant collagen products. Numerous domestic companies have accelerated the development of recombinant collagen injectables to meet the demand for safe and effective aesthetic products. According to the "Regulations on the Supervision and Administration of Medical Devices" and the "Medical Device Classification Catalog," recombinant collagen injectables are classified as Class III medical devices and are subject to stringent regulations to ensure safety and efficacy. As of now, only one such product has been approved in China, but with ongoing technological advancements, more reliable recombinant collagen aesthetic products are expected to enter the market, offering consumers additional choices.

Recombinant Type III collagen can reduce skin photoaging caused by ultraviolet (UV) radiation

Experimental Methods:

A skin damage animal model was created through UV-induced photoaging, and recombinant humanized Type III collagen was used as a bioactive material. It was implanted in vivo to study its biological effects and compared with saline and non-crosslinked hyaluronic acid. During the 8-week experiment, non-invasive and dynamic monitoring of animal skin conditions was conducted. At the end of the animal experiment, skin status was assessed using histological observation, specific gene expression, and other molecular biological methods.

Experimental Results:

Recombinant humanized Type III collagen can mitigate UV-induced skin photoaging by:

- Reducing abnormal thickening of the epidermis and dermis
- Increasing secretion of Type I and Type III collagen
- Remodeling the extracellular matrix, etc.

■ **Medical Dressings (Class II Medical Devices)**

Recombinant collagen medical dressings combine medical and cosmetic features and are primarily used for skin repair in aesthetic medicine, skin injuries, chronic eczema, and allergy recovery. These products are subject to more stringent regulations than traditional cosmetics, with stricter requirements for safety and efficacy. The target consumer groups include individuals with skin diseases, those undergoing minimally invasive cosmetic procedures, and those seeking skincare benefits. Due to the good safety profile and stable effects of recombinant collagen medical dressings, they have a high repurchase rate in the expanding aesthetic market.

Recombinant Triple-Helix Collagen Dressings Accelerate Microneedle Wound Healing

Experimental Methods:

Recombinant triple-helix collagen dressings were tested for safety, repair effects on light-induced damage, and microneedle damage in New Zealand rabbits and rat models.

Experimental Results:

- Recombinant triple-helix collagen dressings are gentle, safe, and non-irritating.
- After 4 days of treatment, these dressings effectively healed damaged dermis by accelerating re-epithelialization and promoting collagen deposition.
- Dressings with varying concentrations of recombinant triple-helix collagen demonstrated a similar rapid epithelialization rate as commercial bovine collagen dressings within 48 hours.

Sources: Public information, literature search, and Frost & Sullivan analysis.

4.2 Applications of Recombinant Type III Collagen — Aesthetic Medicine

As the production process for recombinant collagen advances, more related products will emerge, boosting the market share of recombinant collagen aesthetic injectables. As the aesthetic consumer base expands, the market for recombinant collagen medical dressings will also grow.

4.2.3 Market Analysis of Recombinant Collagen in the Aesthetic Medicine Sector

■ Aesthetic Injectables

Injectable aesthetic treatments are favored by consumers due to their safety, short recovery times, and affordability. The three main bioactive ingredients in these injectables are hyaluronic acid, botulinum toxin, and collagen. In China, collagen-based aesthetic injectables, particularly recombinant ones, were introduced later than hyaluronic acid and botulinum toxin, resulting in lower market penetration historically. Before 2021, there were no recombinant collagen aesthetic injectables available in China. As the recombinant collagen production process matures, more products are expected to enter the market. Given its unique biological properties, recombinant collagen-based injectables are anticipated to capture a larger market share.

According to Frost & Sullivan's analysis, from 2019 to 2023, the market size of China's medical aesthetics injectables in the retail segment increased from 30.9 billion RMB to 67 billion RMB, with a CAGR of 21.4%. It is expected to reach 147 billion RMB by 2027.

In 2023, the market size of recombinant collagen medical aesthetics injectables in the retail segment was 4.3 billion RMB, accounting for 6.4% of the overall medical aesthetics injectable retail market size and 64.1% of the collagen medical aesthetics injectable retail market size. By 2027, the market size of recombinant collagen medical aesthetics injectables is projected to reach 14.3 billion RMB, accounting for 9.8% of the overall medical aesthetics injectable retail market size and 85.5% of the collagen medical aesthetics injectable retail market size.

■ Medical Dressings

Medical dressings are auxiliary therapeutic products used for medical surgeries, aesthetic treatments, wound care, chronic eczema, and skin repair after allergic reactions, excluding consumables such as gauze.

According to Frost & Sullivan's analysis, from 2019 to 2023, the market size of China's medical dressing in the retail segment increased from 14.3 billion RMB to 48 billion RMB, with a CAGR of 57.8%. It is expected to reach 119.2 billion RMB by 2027.

In 2023, the market size of recombinant collagen medical dressings in the retail segment was 11 billion RMB, accounting for 23.0% of the overall medical dressing retail market. By 2027, the market size of recombinant collagen medical dressings is projected to reach 34.6 billion RMB, accounting for 29.0% of the overall medical dressing retail market.

Fig. 2019-2027E China Aesthetic Injectable Market Size (Retail End)

Time Period	CAGR		
	Recombinant Collagen	Collagen	Aesthetic injectables
2019-2023	NA	26.5%	21.4%
2023-2027E	35.4%	26.0%	21.7%

Unit: hundred million RMB

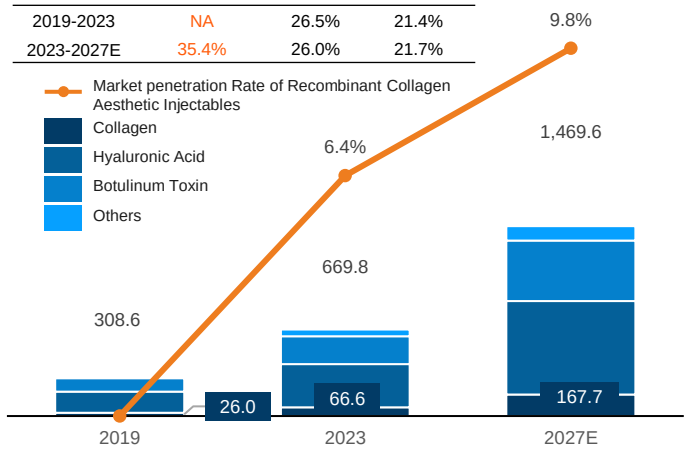
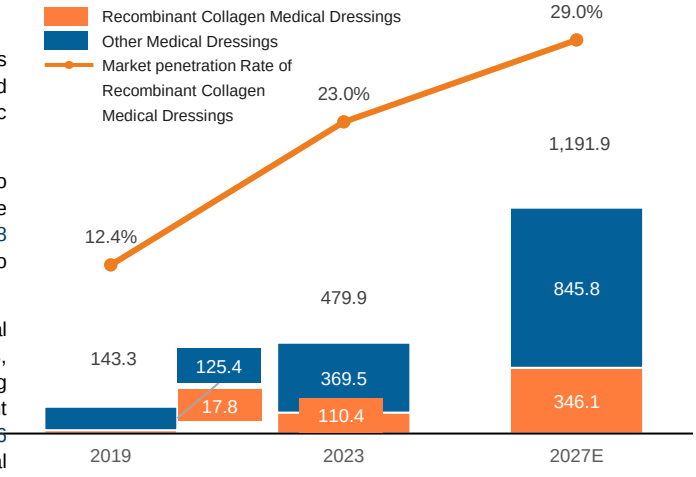


Fig. 2019-2027E China Medical Dressing Market Size (Retail End)

Time Period	CAGR		
	Others	Recombinant Collagen	Total
2019-2023	31.0%	35.3%	57.8%
2023-2027E	23.0%	25.5%	33.1%

Unit: hundred million RMB



Sources: Public information, and Frost & Sullivan analysis.

4.2 Applications of Recombinant Type III Collagen — Efficacy-Driven Skincare

Collagen is a natural moisturizer used in Efficacy-Driven Skincare. Recombinant technology optimizes amino acid sequences, improves purity, exposes integrin binding sites, enhances absorption, and boosts safety.

4.2.4 Applications of Recombinant Type III Collagen in Efficacy-Driven Skincare

To address specific skin concerns, efficacy-driven skincare products use gentle formulas and active ingredients like collagen, hyaluronic acid, and plant extracts to promote skin health and offer multiple benefits.

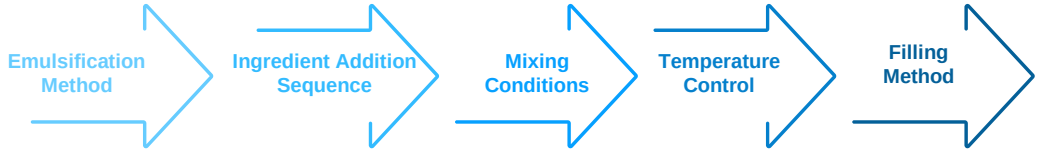
Collagen, a natural moisturizer and repair agent, is widely incorporated into various cosmetic formulations. Recombinant collagen technology enables the optimization of collagens' amino acid sequences, improving purity, integrin binding site exposure, transdermal absorption, and overall safety.

Fig. Types of Recombinant Type III Collagen Skincare Products

■ **Focus of the issue and Solutions for Recombinant Type III Collagen in Efficacy-Driven Skincare**

Focus of the issue	Solutions
High temperatures during skincare product processing can affect their functionality.	
High temperatures can accelerate chemical reactions in skincare products, causing the degradation or loss of activity of certain active ingredients such as vitamins, collagen, and hyaluronic acid. For products containing temperature-sensitive ingredients, high temperatures can lead to oil-water separation in emulsions or creams, affecting their consistency and durability.	• **Optimization of Preparation Process:** Optimizing various stages of the cosmetic preparation process allows for better temperature control, and adjusting the order of ingredient addition can help minimize the loss of active ingredients. • **Improvement of Raw Materials to Enhance Stability:** Enhancing the stability of recombinant Type III collagen raw materials can help maintain their biological activity at higher temperatures.

Fig. Skincare Product Preparation Process



Focus of the issue	Solutions
Low transdermal absorption rate of active ingredients	
When skincare products are applied to intact skin, the active ingredients passively diffuse through the stratum corneum, epidermis, and dermis, with each layer playing different roles. Due to the tightly packed structure and hydrophobic barrier properties of the stratum corneum, the rate-limiting step in transdermal absorption is primarily passive diffusion through this layer. It is generally accepted that the skin's absorption of substances with molecular diameters greater than 500 Daltons (Da) is very limited.	• By controlling the size of active ingredient carriers and creating smaller particle "nano-emulsions," product absorption and stability are improved, while also enhancing skin feel. • In addition, reducing the molecular weight of collagen, through fragment selection or hydrolysis, makes it easier for the skin to absorb.

Sources: Public information, literature search, and Frost & Sullivan analysis.

4.2 Applications of Recombinant Type III Collagen — Efficacy-Driven Skincare

With growing demand for better skin and anti-aging solutions, the market for collagen-based efficacy-driven skincare in China continues to expand, with recombinant collagen seeing consistent increases in market penetration.

4.2.5 Market Analysis of Recombinant Collagen in Efficacy-Driven Skincare

As unhealthy lifestyles, prolonged exposure to blue light from electronic devices, and environmental pollution become more prevalent, more people are experiencing skin issues. These issues include sensitivity, premature aging, chronic eczema, and allergies. These conditions can lead to symptoms like stinging, itching, burning, dryness, peeling, bumps, and hives, driving increased demand for efficacy-driven skincare products. Additionally, these products are not only providing solutions for consumers with specific skin concerns but are also becoming increasingly popular among those with generally healthy skin.

According to Frost & Sullivan's analysis, from 2019 to 2023, the market size of China's collagen functional skincare in the retail segment increased from 3.08 billion RMB to 14.43 billion RMB, with a CAGR of 47.1%. It is expected to reach 78.97 billion RMB by 2027, with a CAGR of 53.0% from 2023 to 2027.

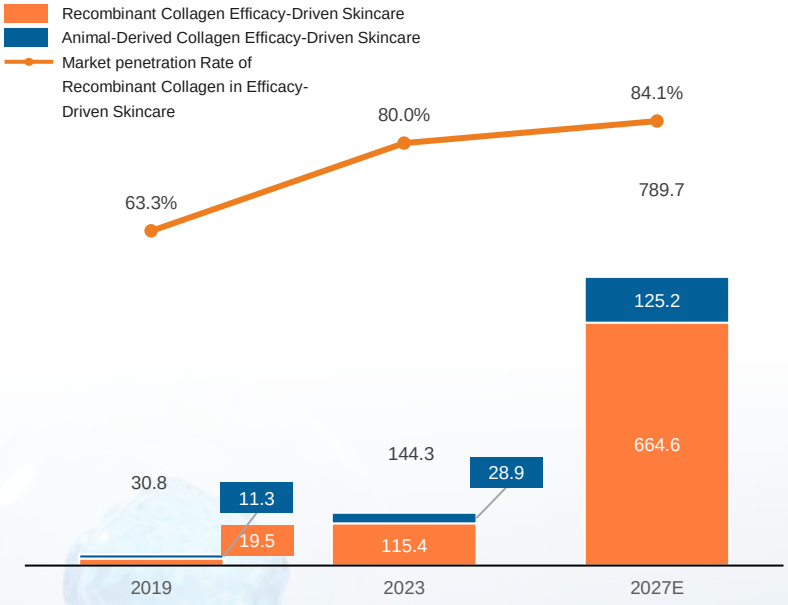
The retail market for recombinant collagen-based functional skincare products grew from 1.95 billion RMB in 2019 to 11.54 billion RMB in 2023, with a CAGR of 55.9%. By 2027, this market is expected to reach 66.46 billion RMB, with a CAGR of 54.9%.

Additionally, the penetration rate of recombinant collagen-based functional skincare within the collagen-functional skincare market increased from 63.3% in 2019 to 80.0% in 2023 and is expected to rise to 84.1% by 2027.

Fig. 2019~2027E China Collagen Efficacy-Driven Skincare Market Size (Retail End)

Time Period	CAGR		
	Animal-Derived Collagen	Recombinant Collagen	Total
2019-2023	26.5%	55.9%	47.1%
2023-2027E	44.3%	54.9%	53.0%

Unit: hundred million RMB



Source: Public Information, Frost & Sullivan Analysis

4.2 Applications of Recombinant Type III Collagen — Other Consumer Fields

In addition to its use in Efficacy-Driven Skincare, the application of recombinant type III collagen has extended to other consumer fields as awareness of its benefits grows.

4.2.6 Application of Recombinant Collagen in Other Consumer Fields

Health Supplements

As early as thousands years ago, ancient Chinese people used natural substances like donkey-hide gelatin and pig skin to supplement "collagen," believing it would improve beauty and health. This focus on "collagen" has continued for nearly a millennium. However, traditional sources of collagen contain relatively low collagen content and are high in fat, reducing their nutritional value and making absorption by the body less efficient.

Today, advanced biotechnological techniques have allowed for the hydrolysis of collagen into smaller collagen peptides, which are absorbed far more efficiently than traditional collagen, maximizing their physiological benefits. Studies show that oral collagen peptides can effectively maintain skin elasticity and health, support joint care, increase bone density, and contribute significantly to vascular health and elasticity.

Personal Care Products

Recombinant collagen, known for its superior moisturizing and repairing properties, is not only effective in facial care but also shows great potential for body and scalp care. As beauty needs continue to evolve, more consumers are seeking holistic beauty experiences that include full-body and scalp care, in addition to facial care.

This growing demand has led to increased popularity for recombinant collagen personal care products. These products nourish and hydrate the body's skin while offering effective solutions for scalp conditions like dryness and itching. Their gentleness and effectiveness make them highly appealing to consumers looking for premium personal care solutions.

Products for pets

According to data from Frost & Sullivan, in 2022, the three primary segments of the Chinese pet market—pet food, pet supplies, and pet medical services—had market sizes of 113.6 billion RMB, 41.6 billion RMB, and 106.2 billion RMB, respectively. Between 2015 and 2022, the compound annual growth rates (CAGR) for these segments were 19.0%, 15.6%, and 17.7%, with pet food being the fastest-growing segment. Frost & Sullivan projects that by 2026, China's pet ownership rate could reach 31.9%, indicating significant growth potential compared to the 70% pet ownership rate in the U.S.

As the Chinese pet market expands and people place increasing importance on pet care, recombinant collagen pet food and care products have begun to emerge.

Source: Public Information, Literature Search, Frost & Sullivan Analysis

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