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Review

Collagen Biosynthesis to Engineered Biomaterials: Molecular Design, Synthetic Strategy, and Biomedical Application

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ABSTRACT: Collagen, a principal component of the extracellular matrix, provides mechanical strength and stability to tissues and organs through its structural organization. Its biocompatibility has established it as a crucial material in biomedical applications such as drug delivery systems, cell culture matrices, and tissue engineering scaffolds. However, the use of animal-derived collagen carries risks of pathogen transmission, which has driven research towards developing synthetic collagen alternatives. Advances in AI-assisted protein engineering are accelerating the design of synthetic collagens and their applications in biomaterials. This review examines collagen's structural characteristics, biosynthesis strategies, biological activities as well as AI-assisting engineering.

Keywords: Collagen; Extracellular matrix; Biomaterial; AI-assisting engineering; Synthetic biology

1. Introduction

Collagen, a principal component of the extracellular matrix (ECM) [1], constitutes ~30% of the human body's total protein content. It represents 70% of the skin's dry weight, and ~90% of the protein in tendons and corneal tissue [2]. The collagen family comprises 28 types, with molecular isomerism underpinning functional and structural diversity (Table 1) [3]. This heterogeneity governs collagen's physicochemical characteristics. Some types of collagen have a triple-helix structural feature (Figure 1). These chains intertwine into a superhelix (Figure 1). The repeating $(Gly\text{-}X\text{-}Y)_n$ motif facilitates tight packing of the helices, where X and Y are frequently proline and hydroxyproline. Hydrogen bonds form between the carbonyl group of the X-position residue in one chain and the amide nitrogen of glycine in an adjacent chain [4], creating a lattice parallel to the polypeptide backbone and perpendicular to the helical axis [5].

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Table 1. The diversity of the collagen family.

Collagen Type	Extraction Difficulty	Function	Category	Collagen Subtypes
Collagen I	No	Provide tensile strength [6]	Fibrillar collagen	Banded fibrils
Collagen II	No	Provide compressive strength and elasticity [7]	Fibrillar collagen	Banded fibrils
Collagen III	No	Provides support in soft tissues and maintains elasticity [8]	Fibrillar collagen	Banded fibrils
Collagen IV	Strong ECM interactions	Provide scaffolding for epithelial and endothelial layers [9]	Non-fibrillar collagen	Network-forming collagens
Collagen V	Strong ECM interactions, abundant in the embryonic stage	Co-assemble with type I collagen in tissues like skin and placenta [10]	Fibrillar collagen	Banded fibrils
Collagen VI	Low abundance, strong ECM interaction	Mechanical support, cytoprotective function, promotion of tumor growth and progression [11]	Non-fibrillar collagen	Beaded microfibrils
Collagen VII	Low abundance, strong ECM interaction	Provide stability to the dermal-epidermal adhesion [12]	Non-fibrillar collagen	Anchoring fibrils
Collagen VIII	Low abundance, strong ECM interaction	Active roles in angiogenesis and ECM remodeling [13]	Non-fibrillar collagen	Short chain collagens
Collagen IX	Low abundance, strong ECM interaction	Support cartilage integrity and stability [14]	Non-fibrillar collagen	FACIT
Collagen X	Low abundance, strong ECM interaction	Regulate matrix mineralization and compartmentalizing matrix components [15]	Non-fibrillar collagen	Fibril-associated collagens
Collagen XI	Abundant in embryonic stage, strong ECM	Regulate collagen fibrillogenesis [16]	Fibrillar collagen	Fibril-forming collagens
Collagen XII	Low abundance, strong ECM interaction	Stabilize type I collagen fibrils [17]	Non-fibrillar collagen	FACIT
Collagen XIII	Transmembrane nature	Function as an adhesion molecule [18]	Non-fibrillar collagen	Transmembrane collagen
Collagen XIV	Abundant in the embryonic stage, strong ECM interactions	Regulate early stages of fibrillogenesis [19]	Non-fibrillar collagen	FACIT
Collagen XV	Low abundance	Structural link between producing cells and connective tissues [20]	Non-fibrillar collagen	Multiplexing
Collagen XVI	Abundant in the embryonic stage, strong ECM interactions	Support interaction of connective tissue cells with their ECM [21]	Non-fibrillar collagen	FACIT
Collagen XVII	Transmembrane nature	Facilitate epidermal-dermal attachment, a niche for hair follicle stem cells [22]	Non-fibrillar collagen	Transmembrane collagen
Collagen XVIII	Abundant in the embryonic stage	Control blood vessel formation [23]	Non-fibrillar collagen	Multiplexing
Collagen XIX	Low abundance, strong ECM interaction	Unknown functions. Suggest the regulation of cardiac extracellular matrix structure [24]	Non-fibrillar collagen	FACIT
Collagen XX	Low abundance, strong ECM interaction	Unknown functions. It may serve as a biomarker of solid tumors [25]	Non-fibrillar collagen	FACIT
Collagen XXI	Low abundance, strong ECM interaction	Unknown functions. May contribute to the extracellular matrix assembly [26]	Non-fibrillar collagen	FACIT
Collagen XXII	Low abundance, strong ECM interaction	Act as a cell adhesion ligand for skin epithelial cells and fibroblasts [27]	Non-fibrillar collagen	FACIT

Collagen XXIII	Transmembrane nature	Unknown functions. An important biomarker for lung cancer [28]	Non-fibrillar collagen	Transmembrane collagen
Collagen XXIV	Low abundance, limited in developing bone, and strong ECM interactions	Promote fibrillogenesis in bone and cornea [29]	Fibrillar collagen	Fibril-forming collagen
Collagen XXV	Low abundance, strong ECM interaction	Promote fusion of myoblasts into myofibers [30]	Non-fibrillar collagen	Transmembrane collagen
Collagen XXVI	Low abundance	Unknown functions. Suggest to support testis and ovary development [31]	Non-fibrillar collagen	FACIT
Collagen XXVII	Abundant in the embryonic stage	Support calcification of cartilage and the transition of cartilage to bone [32]	Fibrillar collagen [33]	Fibril-forming collagen
Collagen XXVIII	Low abundance	May contribute to neuron protection and support [34]	Non-fibrillar collagen	Fibril-forming collagen

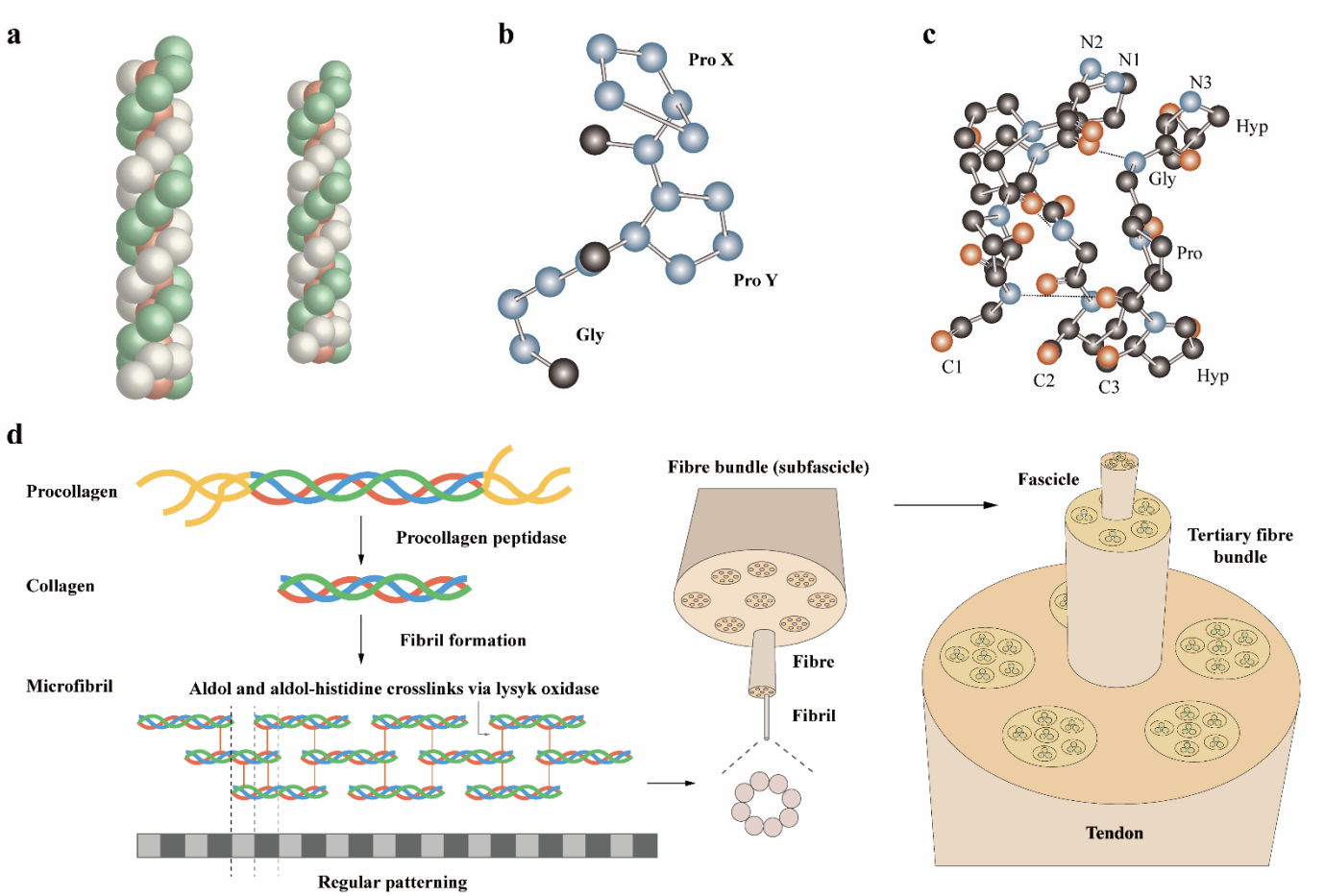

Figure 1. Fibrillar collagen assembly and the collagen triple helix: (**a**) Structure of triple helix, consisting of the repeating (ProHypGly)4-(ProHypAla)-(ProHypGly)5 sequence [35]. (**b**) ProX-ProY-Gly chain in collagen triple helix [36]. (**c**) Three strands in the collagen triple helix stagger together and form a ladder-like pattern of hydrogen bonds. (**d**) The biosynthesis of collagen begins with procollagen. Collagen molecules assemble into microfibrils in the extracellular matrix [37,38].

Collagen plays a pivotal role in mediating interactions between cell and the ECM [39]. A triple-helical domain characterizes fibrillar collagen. Other collagen types, such as fibril-associated collagen with interrupted triple helices (FACIT), demonstrate the interspersion of triple-helical domains within non-collagenous (NC) domains. These NC domains are crucial for structural assembly and confer biological activity to collagen [40].

1.1. Fibrillar Collagen

Fibrillar collagen exhibits a hierarchical structure wherein parallel staggered collagen molecules self-assemble into fibrous nanostructures that further aggregate into higher-order assemblies [41]. Synthesis begins with the production of procollagen precursors containing carboxy-terminal propeptides and signal sequences that direct trafficking to the rough endoplasmic reticulum (ER) [42]. Within the ER, propeptides undergo hydroxylation of lysine and proline residues by lysyl and prolyl hydroxylases, respectively [42]. Proline and hydroxyproline play a crucial role in stabilizing the triple helix [43,44]. Following lysyl hydroxylation and O-linked glycosylation, α-chains trimerize into procollagen, a process initiated by the C-propeptide domain. This domain recognizes α-chains via a specialized mechanism, forming a stable core that drives triple helix assembly [45–47].

The formation of collagen microfibers involves the nucleation, organization, and unidirectional elongation of short primary nanofibers (Figure 1) [48,49], which subsequently merge to form microfibrils exhibiting increased longitudinal and axial dimensions [50,51]. These supramolecular assemblies undergo stabilization via covalent crosslinking [52], a process initiated by extracellular lysine oxidases that catalyze the oxidative deamination of lysine residues in target peptides [53]. Within the microfibrils, the N- and C-termini of adjacent collagen monomers interact and are covalently cross-linked by lysyl oxidase, further enhancing structural stability [53,54]. Ultimately, the assembly of collagen microfibers depends on the complex interplay of chemical and physical interactions among its components [55].

1.2. Non-Fibrillar Collagen

The collagen family also includes non-fibrillar members, such as network-forming collagen IV, which assemble into sheet-like networks rather than fibers [56]. These collagen IV networks have been vital to the evolution of multicellular organisms [57]. Collagen IV differs from fibrillar collagen in two key aspects of its higher-order assembly [58]. First, the C-terminal non-collagenous (NC1) domain of collagen IV is retained during assembly and plays a central role in network formation [9,59]. Second, collagen IV features a sequence with a discontinuity in the (Gly-X-Y)n repeat, where glycine is absent or replaced in one of the three residues. This disrupts the formation of continuous triple-helical regions and creates local structural instability [60], a feature also seen in other non-fibrillar collagens [4].

The assembly of collagen IV scaffolds is a complex process. Intracellular enzymes collaborate to construct the heterotrimer, but the assembly into a three-dimensional (3D) scaffold occurs extracellularly. After secretion, protomers align via their NC1 and 7S domains, forming critical junctions at the protofibril ends. The triple helix then undergoes superhelical formation through lateral interactions [61]. During this process, functional molecules are incorporated into the collagen triple helix by embedding binding sites along the protofibril length. These network-forming collagens act as "smart" scaffolds, especially as components of the basement membrane, supporting the development and function of multicellular tissues [9].

1.3. Biological Function of Collagen

Collagen is vital in modulating a range of signaling pathways essential for development, regeneration, and tissue repair, thus maintaining tissue homeostasis. Its interaction with cells is primarily mediated by specific receptors facilitating bidirectional transmission of mechanical and biochemical signals through cytoskeleton-mediated processes (Table 2). Key receptors include integrins and discoidin domain receptors (DDR1 and DDR2) [62,63].

Integrins are heterodimeric receptors present on nearly all cell types and serve as major mediators for extracellular matrix components, including collagen. They play pivotal roles in regulating cell signaling, migration, survival, and differentiation [63,64]. Four collagen-binding integrins, α1β1, α2β1, α10β1, and α11β1, have been identified within the integrin α1 domain subgroup [65–67]. Although they share the function of collagen receptors, they are expressed in different cell types and mediate distinct biochemical signals. For example, α1β1 integrin interacting with collagen triggers biological responses such as Grb2 recruitment, MAPK activation for cell proliferation, FAK phosphorylation facilitating fibroblast-to-myofibroblast differentiation, and activation of the Shc-mediated pathway in skin regeneration.

Receptor tyrosine kinases, especially DDR1 and DDR2, are also activated upon collagen binding [68,69]. DDR1 is mainly expressed in epithelial cells, while DDR2 is found in fibroblasts and mesenchymal cells. Unlike integrins, DDRs mediate ECM signaling unidirectionally. DDR2's interaction with collagen II is indirectly regulated by integrin/cytokine pathways and AGE-mediated signaling [70,71]. DDR2-bound collagen in the ECM activates the JNK/MAPK and PI3K/Akt pathways, influencing cell proliferation, survival, and gene expression. DDR1 binding to collagen activates JNK, NF-κB, p38, ERK1/2 MAPKs, and PI3K/Akt signaling pathways. DDR1 inactivation leads to interactions with E-cadherin, promoting cell-cell contact. Both collagen-mediated cell-matrix communication and collagen-independent cell-cell interactions influence the triggering of diverse signaling pathways through DDRs [72].

Table 2. Receptors related to Collagen.

Receptor	Type	Distribution	Biological Regulation	Ligand Binding Specificity
Integrins	α1β1	Fibroblasts, Mesenchymal tissues	Wound healing; Regulates the proliferation of living cells, MMP expression, and collagen synthesis; Fibroblast to myofibroblast differentiation; Invasion and growth of hepatocellular carcinoma	Collagens I, III, IV, IX, XIII, XVI, and the collagen IV chain–derived peptide arresten [73–75]
	α2β1	Platelets, epithelium, Fibroblasts, and Mesenchymal tissues	Platelet adhesion to collagen; Hepatocellular carcinoma invasion and growth; Wound healing	Collagens I, III, IV, V, XI, XVI, and XXIII [76–78]
	α10β1	Cartilage and Chondrocytes	Chondrogenic differentiation; Cartilage repair, Skeletal growth	Collagen II and IX [78]
	α11β1	Periodontal ligaments	Wound healing; Cell migration; Mediates the contraction of collagen lattices; Myofibroblast differentiation	Collagen I and XIII [79,80]
Receptor Tyrosine Kinases (DDR)	DDR1	Epithelial cells, Smooth muscle cells, Fibroblasts, Oligodendrocytes and Macrophages	Development and growth of organs; Cell proliferation, survival, homing, and colonization; Inhibits tumor growth	Collagen I–V [81]
	DDR2	Chondrocytes	Development and growth of organs; Development of bone and cartilages; Pathological process of arthritis, wound healing, dwarfism, and tumor	Collagen I–III, and V[81]
Immunoglobulin Receptor	GPVI	Megakaryocytes and Platelets	Wound healing	Collagen I–III [82]
	OSCAR	A wide range of myeloid cells	Osteoclast growth induction for bone resorption; Osteoclast differentiation	Collagen I and II [83]
Leukocyte Receptor Complex (LRC)	LRC	Immune cells	Autoimmunity; Antiviral immunity; Graft tolerance; Regulates osteoclast differentiation.	Collagen I and III [84]
Other Receptors	Fibronectin	Extracellular matrix, Plasma, and Cell surface	Tissue growth; Wound repair; Fibroblast migration; Nerve regeneration stabilization; Extracellular matrix and embryogenesis; cell-to-cell adhesion	Collagen I and III [85]
	Vitronectin	Extracellular matrix, Blood serum, Platelets, and Bone.	Cell proliferation; Adhesion; Immune defense; Hemostasis; Fibrinolysis	Collagen I [86]
	uPARAP	Mesenchymal cell surface, Osteocytes, and Osteoblasts	Fibroblast migration; Primary adhesion of collagen to fibroblasts	Collagen I, II, IV, and V [87]

GPVI: Glycoprotein VI; OSCAR: Human osteoclast-associated receptor.

2. From Native Collagen to Protein Engineering

The isolation of collagen from natural tissues laid the foundation for its application in biomedicine, yet the inherent limitations of native collagen extraction have catalyzed the evolution toward engineered alternatives. Pioneering work by Lister and Macewen utilizing sheep intestinal collagen sutures [4,88] established collagen's biocompatibility, while its subsequent use as cell culture matrices [89] revealed structural dependency on tissue-specific supramolecular assemblies [90,91].

Traditional extraction protocols predominantly utilize mammalian sources like bovine, pig skin, and rat tail tendon, and marine byproducts like fish through acidic/alkaline hydrolysis or enzymatic digestion [92–94]. Collagen from bovine Achilles tendon [95,96] and porcine skin [97] dominated early biomaterial development [98–100].

Acid and alkaline extraction methodologies inevitably perturb these critical structural determinants through three primary mechanisms (Figure 2). First, thermal denaturation at temperatures exceeding 40℃ induces unwinding of the triple helix into disordered random coils, leading to an 85% reduction in tensile strength compared to native fibrillar collagen [90]. Second, enzymatic hydrolysis using proteases such as pepsin preferentially cleaves non-helical telopeptide regions, generating fragmented polypeptides (3–6 kDa) with compromised cell-binding RGD motifs essential for integrin-mediated signaling [101–103]. Third, the hydrolysis of lysyl oxidase-catalyzed pyridinoline crosslinks, as evidenced by the decrease in Young's modulus [99].

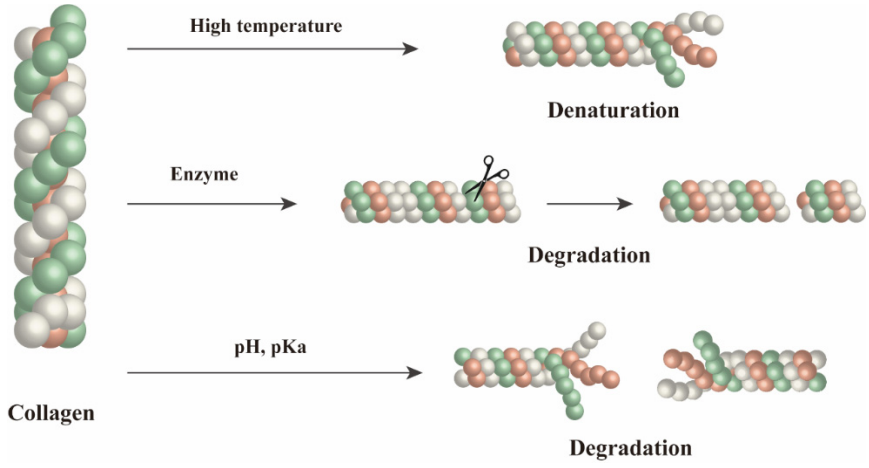

Figure 2. Structural Degradation During Natural Collagen Extraction: There are three principal mechanisms that compromise collagen's structural integrity during the extraction process: thermal denaturation, enzymatic hydrolysis, and cross-link disruption.

To overcome these limitations, collagen mimetic peptides (CMPs) have emerged as engineered alternatives that recapitulate core triple-helical motifs while enabling programmable functionality. Recent advances demonstrate that CMPs can be rationally designed to resist thermal denaturation through strategic incorporation of unnatural amino acids or covalent cross-linking [104]. Notably, π-system end-capping strategies stabilize short CMPs with only 3–6 repeats, achieving melting temperatures up to 76 °C [105]. Furthermore, functional domains (e.g., GFOGER for integrin binding) can be embedded without disrupting fibril morphology, restoring cell-adhesion activity lost in enzymatically hydrolyzed collagen fragments [106,107]. These approaches collectively enable the synthesis of chemically complex, well-controlled collagen mimetic biomaterials.

3. Biological Synthesis of Recombinant Collagen

The drawbacks of native collagen extraction, such as the risk of pathogen transmission, immunogenicity, structural heterogeneity, and limited scalability, have prompted a shift toward recombinant collagen production [108] (Table 3). Recombinant strategies enable control over amino acid sequence, post-translational modifications (PTMs), and molecular architecture, allowing the fabrication of collagen with tunable properties (Figure 3). *Escherichia coli* is widely favored among expression systems for its rapid proliferation, low production cost, and high-density fermentation capability [109]. However, the lack of endogenous prolyl-4-hydroxylase (P4H) in *E. coli* impairs hydroxyproline synthesis, thereby limiting triple-helix thermal stability [110]. To overcome this, researchers have implemented heterologous coexpression of P4HA and P4HB from *Caenorhabditis elegans*, increasing hydroxyproline content to 15% and raising the collagen melting temperature (T_m) by approximately 7 °C [111–113]. Further enhancements involve

rational sequence engineering, extending Gly-X-Y repeats from 20 to 40 units to improve interchain packing, and the fusion of elastin-like polypeptides (ELPs) to the N-terminus [114], enabling temperature-responsive trimerization with over 80% efficiency at physiological temperature [115].

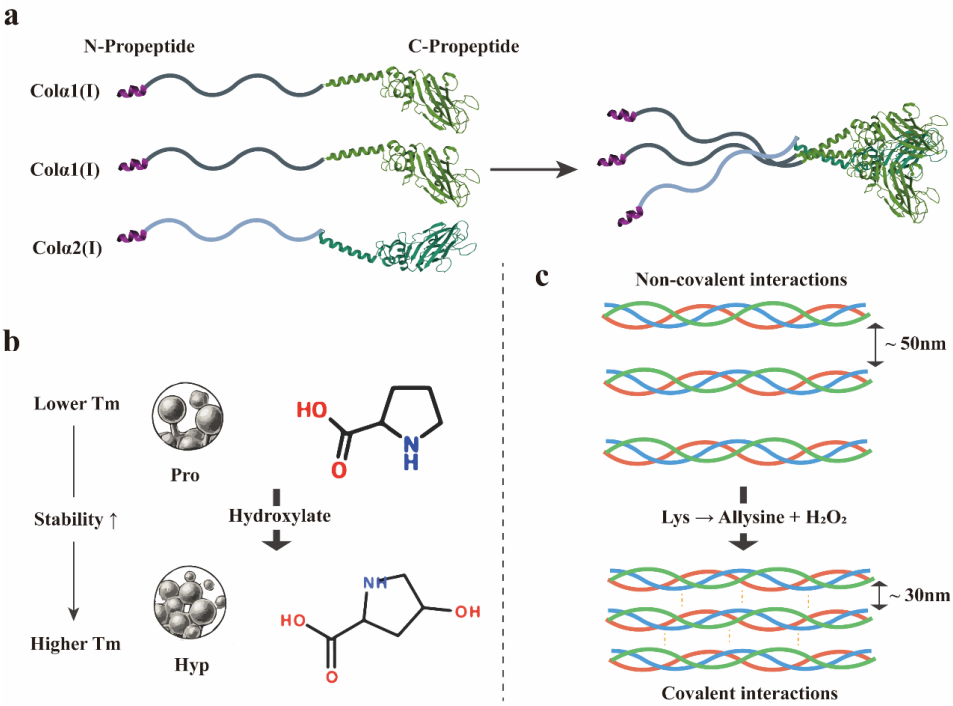

Figure 3. Protein Engineering Strategies for Collagen Stabilization: (**a**) C-propeptide-mediated heterotrimeric assembly of type I collagen. (**b**) Hydroxyproline-mediated stabilization of triple-helix thermodynamics. (**c**) Enzymatic crosslinking enhances fibrillar shear resistance.

Yeast expression systems, particularly *Pichia pastoris*, offer a more conducive environment for collagen biosynthesis with appropriate PTMs. Coexpression of human P4HA1/P4HB achieves hydroxyproline levels up to 88% of native collagen [116,117] while optimized dual-promoter constructs allow stoichiometric expression of pro-α1 (I) and pro-α2 (I) chains for heterotrimer formation [118]. Nonetheless, purification of heterotrimers at >65% remains a technical challenge. Industrial-scale fed-batch fermentation faces challenges in dissolved oxygen management, where maintaining >30% saturation without oxidative stress requires fine-tuned bioreactor control. Additionally, the production process in yeast systems often requires strict control of methanol induction, pH stability, and osmolarity to avoid protein aggregation and ensure correct folding. These parameters are dynamically regulated through feedback-controlled fermentation systems integrating real-time methanol sensing, DO and pH monitoring, and osmotic pressure balancing. Strategies such as pulse-wise methanol feeding (1.0–2.0 g/L), pH stabilization at 5.0 ± 0.1, and osmolyte supplementation (e.g., sorbitol, betaine) have enhanced solubility and reduced aggregation [117]. Additionally, additives like sodium pyruvate can boost TCA cycle flux, increasing collagen yield by over 20% and shortening induction time. Industrial-scale systems often employ high-density fed-batch protocols (>150 g DCW/L) with optimized agitation-oxygen transfer to maintain protein quality [119]. Use of engineered strains with enhanced secretory pathways, or those co-expressing molecular chaperones, has shown promise in improving the yield and solubility of full-length collagen proteins. Recent progress also includes the coexpression of lysyl oxidase homologs (e.g., LOXL2) [120] to introduce enzymatic crosslinking and targeted N-glycosylation at Asn-X-Ser motifs to improve proteolytic resistance and in vivo stability [121]. Moreover, in vitro hydroxylation using purified P4H enzymes has emerged as a complementary strategy for microbial-expressed collagen, allowing post-expression modification of Hyp content without the need for endogenous enzymatic activity. (Figure 4) Mammalian expression systems such as CHO and HEK293 cells represent the advancements for producing recombinant collagen with near-native fidelity, particularly in PTMs and structural assembly [122–124]. The production cost remains higher than that of microbial systems, limiting application in high-volume products such as scaffold matrices. Emerging alternatives include stem cell-derived extracellular matrix secretion without genetic modification, wherein extracellular niche modulation induces the synthesis of human ECM collagen (hCol) with native-like molecular weights (α1 ~132 kDa; α2 ~122 kDa),

glycosylation profiles, and fibrillar periodicity [124]. This strategy bypasses transgene-associated regulatory hurdles and offers immunologically safer products. Regarding function and safety, the residues of terminal peptides and non-human glycosylation may trigger an immune response. Studies have been conducted to remove N/C-terminal propeptide and telopeptide from recombinant type I collagen, and the endotoxin is controlled within 0.05 EU/mg to ensure the function and safety [125,126].

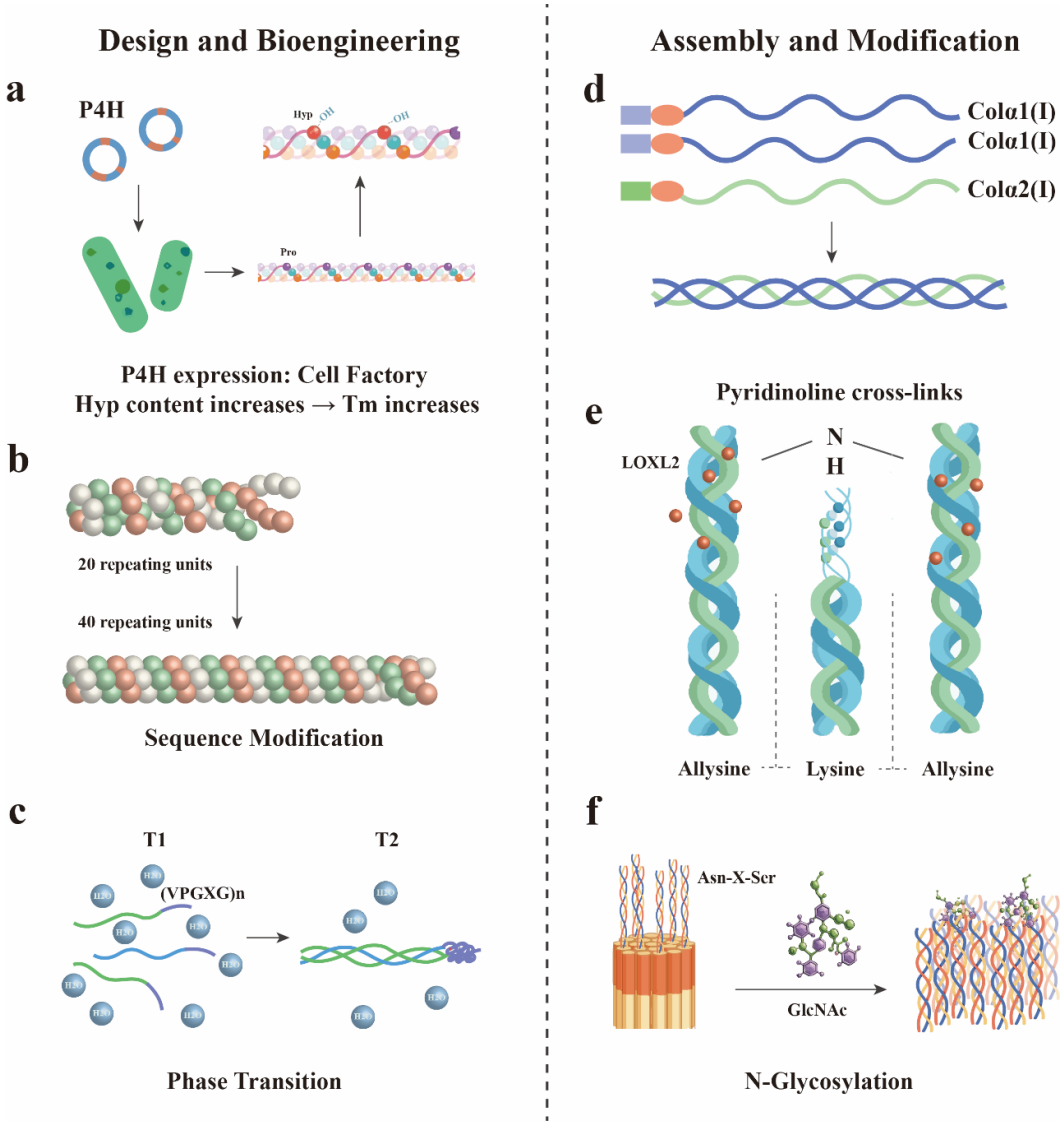

Figure 4. Engineering Strategies for Collagen Biosynthesis. Design and engineering: (**a**) Cell factory for P4H expression; (**b**) Sequence Modification; (**c**) ELP phase separation. Assembly and Modification: (**d**) Chain Stoichiometry Control; (**e**) LOXL2-Mediated Crosslinking; (**f**) Directed N-Glycosylation.

Complementing these bioproduction systems, synthetic biology and AI-guided protein design rapidly advance collagen engineering. Platforms such as ColDiff and ColGen-GA apply diffusion-based modeling and genetic algorithms to design stable, self-assembling collagen variants with tailored biofunctions [127]. AI-assisted strategies in collagen sequence design, structure prediction, and assembly optimization are illustrated in Section 4. These tools enable the rational construction of collagen domains for diverse biomedical applications [128]. Beyond domain-level sequence design, AI-driven simulation platforms now integrate structural dynamics and protein interaction predictions to assess fibrillogenesis potential or improve integrin-binding affinity.

Collectively, recent breakthroughs such as the development of self-assembling recombinant collagen hydrogels without chemical crosslinkers [129,130], high-temperature-resistant triple helix structures stabilized by cold-adapted chaperones, and coexpression of folding enhancers have substantially addressed many technical barriers [131,132]. However, challenges remain in ensuring batch consistency, achieving uniform post-translational modifications, and meeting regulatory standards for clinical application.

Table 3. The fabrication and biosynthesis of collagen.

Type of Collagen	Host	Yield	Comments	Concerned Contaminants
Natural Collagen	–	–	Obtained by enzymatic action; Immunogenicity, pathogen transmission issues; Human collagen extraction is limited and expensive	Residual host proteins; chemical residues; pathogenic microorganisms [92]
Recombinant Human Collagen	*E. coli*	90 mg/L	Coexpression with mimivirus prolyl and lysyl hydroxylases	Endotoxins (from *E. coli* LPS); media residues [113]
	P. pastoris	0.6 g/L	Coexpression with human prolyl hydroxylases in a bioreactor with constant oxygen supply	Cell wall components (β-glucans, Mannans); media residues [117]
	Mammalian cells (293-EBNA)	0.5–80 mg/L	Coexpression with P4H subunits is not required except for collagens X and VIII expression. Low yields of collagen V heterotrimers	Mycoplasma contamination; Viral contaminants [121,127]
	Plants (tobacco)	30 mg/kg	Coexpression with P4H subunits to obtain hydroxylated collagen	Mycotoxins and contaminants from fungal infections; heavy metals; pesticides/herbicides [114,116]
	Transgenic maize seeds	4 mg/kg; 12 mg/kg	Co-expressed with/without both the α- and β-subunits of a recombinant human P4H (rP4H).	
	Drosophila melanogaster S2 cells	10–50 mg/L	Production of collagen I and IX heterotrimers	Mycoplasma contamination; distinct glycosylated products [133,134]

4. AI-Assisted Design and Modeling of Collagen

4.1. Advances in AI-Driven Protein Engineering

Recent advances in deep learning have enabled the *de novo* design of synthetic collagen with programmable properties. ProtSeed, a sequence-structure co-design framework, has shown a 3.2-fold increase in heterotrimer assembly efficiency over random sequences by optimizing charge complementarity and steric compatibility at glycine-X-Y junction [135]. Progress in deep learning has transformed collagen structural prediction and design. The AlphaFold Multimer model has shown excellent performance in predicting protein complex structures, with prediction accuracies of 67% and 69% at the interface between hetero and homo oligomers, respectively, when tested on 4433 protein complexes [136]. The progress in geometric deep learning and the application of models have enhanced our ability to predict and design collagen structures, leading to the development of new collagen-based materials and therapies with tailored properties.[137–139]

4.2. AI-Assisting Design of Collagen Variants

Generative artificial intelligence (AI) technologies have opened new window for the *de novo* design of collagen. Tools based on autoregressive models and Transformer architectures [131–133], such as ProteinMPNN [140] and ESM-IF [141], have enhanced sequence design precision by integrating geometric features, such as torsion angles and backbone vectors, and geometric vector perceptrons (GVPs), particularly excelling in tasks requiring sub-nanoscale control, such as collagen fibril assembly [142]. For instance, the diffusion model ColDiff, combined with a supervised learning strategy, extracts sequence features from human collagen multi-omics data to generate collagen-mimetic peptides (CMPs) with GXY repeat structures, achieving a Pearson correlation of up to 0.95 (natural collagen) and 0.8 (synthetic CMPs) between predicted and experimental melting temperatures (T_m) [143]. Additionally, the synergistic application of genetic algorithms (GA) and deep learning, like ColGen-GA, enables rapid generation of homotrimeric type I collagen sequences (1000 sequences in 8 h), with T_m prediction errors less than 5%, outperforming traditional molecular dynamics simulations [144].

Sequence design based on generative models requires modular strategies combined with experimental validation to optimize biological functionality. The adhesion module guides self-assembly into periodic banded fibers through hydrophobic or electrostatic interactions, while the functional module, based on Streptococcus Scl2 collagen-like protein fragments, introduces osteoblast-binding domains without disrupting fiber morphology [145]. Coarse-grained simulations and atomic modeling elucidate the hierarchical assembly of collagen triple helices into fibrils via gap-overlap stacking, consistent with experimental observations [146,147]. Experimental validation shows that such synthetic collagen achieves tensile strength approaching 50 MPa comparable to natural type I collagen and promotes osteogenic precursor cell differentiation, increasing alkaline phosphatase activity by over 2-fold [148]. Computational design strategies (e.g., reinforcement learning) enable the generation of collagen-mimetic peptides (CMPs) with high self-assembly propensity, though experimental validation remains critical [149]. Short CMPs can form hydrogels at low concentrations, while long-chain variants require proline hydroxylation (up to 90% in yeast systems) for conformational stability [143], highlighting the necessity of post-translational modifications in biomimetic design.

4.3. AI-Assisting Optimization of Collagen Protein

AI technologies enable quantitative optimization of collagen material properties through high-throughput screening and molecular engineering strategies. The ColGen-GA framework identifies key GXY triplets contributing to thermal stability by analyzing millions of generated sequences: sequences containing $(GPO)_{14}$ exhibit the lowest ΔT_m values (T_m reduction of 3 °C) [144]. Experimental validation further demonstrates that AI-designed recombinant CMPs achieve secretion efficiencies of 0.1–0.2 mg/mL in *Pichia pastoris*, with characteristic CD spectral peaks at 220–222 nm and a 40% improvement in cell adhesion efficiency compared to traditional collagen. Moreover, AI-driven optimization of collagen extraction processes, such as enzyme concentration, temperature, and pH value, reduces energy consumption by 40% and waste by 45%, while enabling collagen recovery from waste materials such as fish scales and bovine hides, showcasing end-to-end efficiency from molecular design to industrial production [150,151]. Future efforts should focus on integrating multimodal data, sequence-structure-function, to enhance model generalizability and leveraging automated experimental platforms, such as iBioFoundry, to complete the "design-synthesis-testing" loop [152].

5. Engineering on Collagen Biomaterials

Contemporary molecular engineering strategies enable the precise customization of collagen biomaterials through domain-specific modifications and advanced crosslinking architectures. These approaches have unlocked transformative potential for synthetic collagens across a range of biomedical applications (Table 4). For example, incorporating cell-binding RGD motifs or MMP-sensitive cleavage sites into collagen scaffolds improves cell-material interactions, a fibrinogen-collagen hybrid hydrogel demonstrated a minimum wound closure rate of 83.3% compared to natural collagen with 69.4% [153]. Similarly, VEGF peptide-functionalized scaffolds enhance vascular regeneration by increasing surface wettability, inducing VEGF receptor phosphorylation, and promoting HUVEC survival and proliferation [154]. These scaffolds, fabricated via simple polymer mixing methods, hold promise for sustaining endothelial cell viability during vascular network formation, potentially improving transplanted tissue survival rates. Beyond regenerative medicine, engineered collagens are facilitating oncology research. Collagen-based 3D tumor models with tunable stiffness recapitulate tumor microenvironment mechanics, enhancing immune checkpoint marker expression and aligning drug response profiles with clinical observations [155,156]. The aberrant crosslinking in pathological matrices revealed by AGE mediated collagen fiber binding in liver cirrhosis, can impair remodeling ability. The fibers crosslinked by AGE form coarse bundles, with a 3-fold increase in diameter and a 60% decrease in macrophage remodeling efficiency, promoting fibrosis progression through cytoskeletal disorder and type II immune polarization [157]. This emphasizes the necessity of precise crosslinking control in engineering supports.

Beyond its conventional biomedical applications, recombinant collagen emerges as a transformative material in intelligent drug delivery systems and tissue engineering. Recent technological advancements have capitalized on collagen's programmable biodegradability to develop stimuli-responsive nanocarriers. pH-sensitive collagen nanocapsules have demonstrated remarkable tumor-specific drug release efficiency, achieving 90% drug release through lysosomal acidity-triggered dissolution at pH 5.5 [158]. Light-responsive elastin-like peptide nanoparticles have enabled spatiotemporal control of targeted cellular delivery through near-infrared-induced phase transitions, enhancing targeting efficacy by two-fold [159].

In the realm of tissue engineering, recombinant collagen has exhibited good biocompatibility and cell activity promotion in skin wound repair applications. The incorporation of recombinant collagen into GelMA (gelatin methacryloyl) has enhanced cellular activity and migration capacity, thereby accelerating wound healing processes [160]. *In vivo* experiments demonstrated that wounds treated with recombinant collagen-modified GelMA achieved an 80% healing rate within 14 days, representing a 1.2-fold improvement compared to untreated diabetic mice. The mechanical and self-healing properties of collagen-based hydrogels can be optimized through the strategic network and chain topology design [161–163]. The mechanical optimization carried out through network topology design now combines with a hyperelastic framework, with a self-recovery rate of>95% after 500 cycles, and a compression tension elastic ratio adjusted to 1.5, approaching natural tissue characteristics [164].

Table 4. Biomedical Applications of Collagen-based Biomaterials.

Applications	Tissue	Origin	Crosslinking Agents	Bioharzard of Crosslinking Agents
Wound dressing	Epidermal and dermal acellular scaffolds	Synthetic/ Natural	1,4-butanediol diglycidyl ether (BDDGE)/ EDC	Contact dermatitis, allergic[120,121]
	Hydrogels based on human-like collagen and carboxyl pullulan	Synthetic	Butanediol-diglycidyl ether (BDDE)	
Tendon Repair	Collagen-glycosaminoglycan scaffold	_	Acrylonitrile butadiene styrene (ABS)	Not an irritant [165]
Treatment of Intervertebral Disc Degeneration and Cartilage Repair	Fibrillized jellyfish collagen and alginate hydrogel	Natural	EDC	Skin irritant [122]
	Self-assembled fibrocartilage	Natural	Lysyl oxidase like-2 (LOXL-2)	Not an irritant [166]
	Type II collagen-hyaluronic acid hydrogel	Synthetic	EDC	Skin irritant [167]
	Type II collagen scaffold/chondroitin sulfate composite gel	Natural	Genipin	LD50: 237 mg/kg (oral route) for mice [168–170]
Drug Delivery	Growth factor-conjugated fibrin microbeads	Synthetic	Genipin	
	Collagen-hydroxyapatite scaffolds/Collagen-chitosan-graphene oxide mixture	Synthetic	EDC/N-hydroxysuccinimide (NHS)	An irritant/harmful by ingestion [171,172]
Treatment of Cardiovascular Diseases	Bovine pericardial	_	Dye-mediated photooxidation	Not an irritant [173]
	Decellularized carotids from a newborn calf	Synthetic/ Natural	Co-crosslinking with procyanidins and glutaraldehyde	Skin irritant; may induce asthma [142]
	Alginategelatin-polysaccharide scaffold	Synthetic	Glutaraldehyde	Skin irritant; may induce asthma [174]
Bone Tissue Engineering	Decellularized osteochondral plug from pigs	Natural	Epigallocatechin-3 gallate (EGCG)	Not an irritant [137]
	Recombinant peptide based on human collagen type I	Synthetic/ Natural	Hexamethylene diisocyanate/ Genipin	Skin irritant/LD50: 237 mg/kg (oral route) for mice [138]
	Collagen-glycosaminoglycan scaffold with/without mineral content	Synthetic	EDC/NHS	An irritant/ harmful by ingestion [139]
Neural Tissue Engineering	Collagen/Heparin sulfate scaffold	_	UV light [175]	_
	Type I collagen from tendons	Natural/ Synthetic	Genipin/Glutaraldehyde	LD50: 237 mg/kg (oral route) for mice /skin irritant; may induce asthma [140,176]
Tissue Regeneration	Type I/II collagen composite scaffold	Synthetic	EDC	Skin irritant [141]
	Collagen scaffold	Synthetic	Sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate	Skin irritant [177]
	type I collagen	Natural	Riboflavin 5′ monophosphate (FMN)	Not an irritant [178]
Treatment of corneal diseases	Enucleated rabbit corneas	_	Rose bengal and green light [179]	_
Dentistry	Demineralized human dentin	Natural	Plant-derived polyphenols [180]	
	Bovine collagen	Natural	Epicatechin	Skin irritant [181]

6. Summary and Perspectives

Collagen, the most abundant structural protein in the human body, has become a cornerstone of biomedical engineering. Its hierarchical architecture, biocompatibility, and multifunctional versatility make it highly valuable. This review traces collagen's evolution from its native biological roles to its applications in engineered biomaterials, focusing on molecular design principles, synthetic strategies, and computational innovations that overcome the limitations of natural collagen extraction. Key advancements include developing recombinant expression systems with enhanced thermal stability and AI-driven design platforms exploring new biomaterials.

The integration of computational design, synthetic biology, and advanced manufacturing is pioneering collagen engineering. Next-generation collagen materials programmable in mechanics, biodegradation, and bioactivity will be transferred into regenerative medicine, oncology models, and smart drug delivery systems. However, achieving this vision requires interdisciplinary progress to align molecular-scale innovation with clinical and industrial requirements.

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Author Contributions

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Ethics Statement

Not applicable.

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Not applicable.

Data Availability Statement

The datasets generated during the current study are available from the corresponding author on reasonable request.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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