

REVIEW

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Structure, extraction, processing, and applications of collagen as an ideal component for biomaterials - a review

Qijue Chen¹, Ying Pei^{1*}, Keyong Tang^{1*}  and Mădălina Georgiana Albu-Kaya²

Abstract

Collagen with a multi-hierarchical architecture exhibits powerful biological performance, thus being developed in biomedical applications as a processing building block. The isolated collagen after extraction from biological tissues can be processed into various forms such as fibrils, scaffolds, membranes, microspheres, hydrogels, and sponges for further use in specific applications. This review briefly discusses the multi-hierarchical structure, powerful biological performances, extraction, and processing approaches of collagen as a natural biomaterial. The processing of collagen including dissolution, self-assembly, cross-linking, and electrospinning, is discussed to show more feasibility for specific applications of collagen composite biomaterials. Further emphasis is directed towards the biomedical applications of drug and gene delivery, as well as tissue repair involving bone, cartilage, vascular, and corneal, along with wound healing. Additionally, there is a focus on the development of flexible sensors and electronic skins (e-skins). Furthermore, the potential challenges and perspectives for the development of collagen-based biomaterials are proposed. In short, collagen-based biomaterials are expected to facilitate sustainable development and the next generation of advanced biomaterial applications.

Keywords Collagen, Structure, Extraction, Self-assembly, Biomaterials

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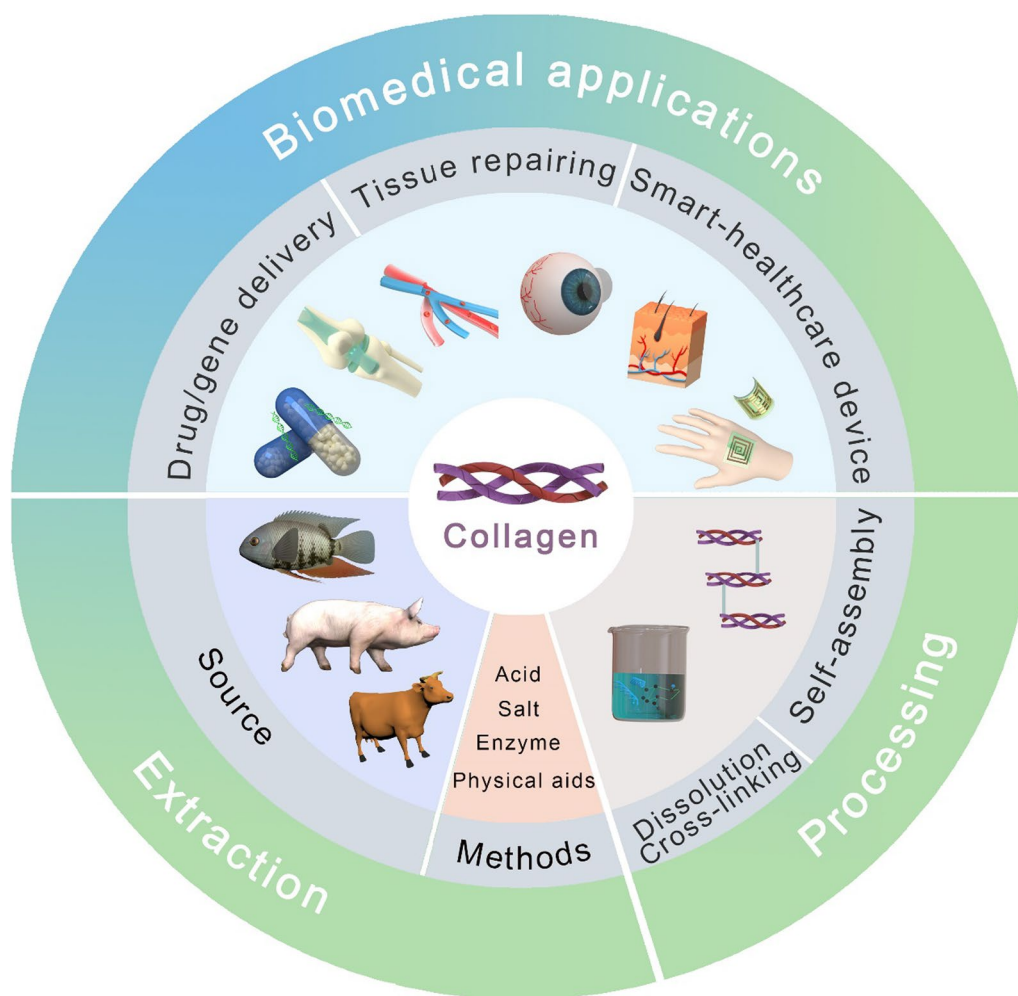
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Graphical Abstract



1 Introduction

Over the years, biomaterials have gained significant interest due to their unique natural characteristics and ability to meet the demands of various applications. These chemically distinct macromolecules include polysaccharides, polypeptides, polynucleotides, polyesters, and polyaromatics [1]. They can be sourced from a wide range of materials that are readily available, eco-friendly, and possess biocompatibility and biodegradability, making them ideal for use in various diagnostic and therapeutic procedures [2]. Among the naturally enriched polymers, collagen stands out as a well-known biomaterial, with rich sources and exceptional biological performances, making it ideal for easing resource constraints, reducing environmental waste, and advancing sustainable progress [3]. Compared to other biomolecules such as cellulose,

chitin, silk, and fibroin, collagen is superior in terms of its numerous processing forms and biological properties.

An inherent aspect of collagen is the multi-hierarchical structure, with the presence of highly ordered structural characteristics from the microscales to macroscales. These characteristics are self-assembled to form nanofibers, microfibers, and fibers that form the basis for biological construction [4]. The complete multi-level structures of collagen exhibit bioactivity and reflect the biosynthesis process, with collagen fibers in tendons serving as structural blocks to sustain essential stretching and contraction in cells. Numerous extracted methods have been developed to obtain collagen with its maintained triple helical structure from abundant natural sources for further processing to achieve high yield and efficiency. Dissolution is available to process collagen into desired

states with other additives. Pei et al. applied liquid exfoliation to obtain a series of 26–230 nm diameter collagen fibers from bovine achilles tendons [5]. In vitro, the self-assembly of collagen was fully investigated, while the mechanism in vivo remains unclear [6]. The isolated fibers that preserved typical structural features of type I collagen were then used as building blocks to fabricate free-standing collagen membranes [5]. Collagen has become increasingly popular as the essential building block for biomaterials due to its excellent biocompatibility, admissible biodegradability, and low immunogenicity [7]. Collagen dominates the extracellular matrix (ECM) in maintaining mechanical strength and bioactivities of connective tissues, and a similar collagen structure has been fabricated based on supramolecular peptides for a biomimetic ECM substitute [8]. Collagen is involved in cell growth, migration, and proliferation, associated with physiological processes of adaptation and tissue regeneration [9]. However, native collagen has poor thermal stability and weak resistance to proteolytic effects. Appropriate crosslinking could confer collagen with ideal physicochemical properties, providing magic to collagen processing. These methods include preparing scaffolds with enhanced stiffness, fabricating hydrogels with sustained adhesion, and preparing microspheres for drug loading (Fig. 2a).

Following successful extraction and processing, collagen-based hybrid materials have demonstrated their potential for use in the field of biomaterials. To fully realize their powerful biological capabilities, collagen-based composites can be processed into various formats such as sponges, microspheres, membranes, and hydrogels for drug delivery, tissue engineering, and clinical therapy (as shown in Fig. 2b) [10]. With its high biocompatibility, collagen can be used as a container for delivering drugs to injured sites. Therefore, it is crucial to ensure the long-term stability of this bio-carrier. Moreover, collagen could participate in the biological activities in the body attributed to the expression function of the encapsulated gene. As a structural component of tissues, collagen exhibits favorable homology for repairing injured tissue; for example, mineralized collagen improves stiffness in bone and cartilage tissue [11], crosslinked collagen hybrids contribute to the vascular formation, bioengineering technologies enhance the mechanical strength and optical properties of collagen-based artificial corneas, and various collagen formats facilitate wound healing and skin regeneration without overuse of antibiotics and antiseptics. Currently, to achieve the promising goal of monitoring human health, collagen can be processed for smart-healthcare devices as a sustainable alternative to bio-waste. Collagen can act as a matrix to link electronic materials for efficient mass transfer, making

flexible sensors or electronic skins (e-skins) a reality. Moreover, with collagen fibers obtained from tannery solid waste, these incredible biomedical applications can achieve high-value utilization by turning waste into a valuable resource [12, 13].

This review endeavors to propose an updated and detailed overview of collagen-based composite biomaterials, including multi-hierarchical structure, powerful biological performances, extraction, and processing approaches. Then, various applications of collagen biomaterials have been fully discussed on drug and gene delivery as vehicles, tissue repairing on bone, cartilage, blood vessels, corneal, and skin, and flexible sensors and e-skins as smart-healthcare devices. Ultimately, the future prospects of collagen-based biomaterials are proposed.

2 Collagen superfamily

2.1 Collagen types

Currently, 29 distinct types of collagen have been identified, featuring variations in the sequence of amino acid residues, morphological structure, distribution, and biophysiological properties [1]. Based on their structural and supramolecular organization, collagen can be primarily classified into fibrillar collagen, microfibrillar collagen, fibril-associated collagen with interrupted triple helix (FACIT), short-chain collagen, anchoring collagen, transmembrane collagen, and basement membrane collagen, as illustrated in Table 1. The collagen superfamilies exhibit diverse functions within specific body tissues, bestowing upon them the potential for a range of biological activities. For instance, fibrillar collagen can provide intricate three-dimensional frameworks for tissues or organs [14], microfibrillar collagen can interact with fibrils and cells [15], and transmembrane collagen can contribute to the development and homeostasis of tissues [16]. Collagen is known for its complexity and variety, encompassing aspects such as structure, splice variants, non-helical domains, and self-assembly functions.

Collagen, which makes up approximately 30% of the body's total protein, exhibits a range of properties due to variations in composition and structure. Each type of collagen contains a triple helical strand and is composed of three identical or different α -chains. Currently, the primary understanding of collagen's hierarchical structure focuses on fibrillar collagen, particularly type I collagen which is the most prevalent. Type I collagen is a trimeric aggregate comprised of two identical $\alpha 1$ (I) chains and one $\alpha 2$ (II) chain in each structural domain. It forms highly-oriented supramolecular aggregates through the arrangement of nearly 1000 amino acids ranging in size of 300 nm in length to 1.5 nm in diameter [40]. Type I collagen has great potential as a structural protein,

Table 1 Collagen types and features

Structural features	Function	Types	Polypeptide chains	Molecular assembly forms	Ref
Fibrillar collagen	To provide three-dimensional frameworks for tissues and organs	I	$[\alpha_1(I)]_2 \alpha_2(I), [\alpha_1(I)]_3$	<i>D</i> -periodic banding space	[14]
		II	$[\alpha_1(II)]_3$		[17]
		III	$[\alpha_1(III)]_3$		[18]
		V	$[\alpha_1(V)]_2 \alpha_2(V), [\alpha_1(V) \alpha_2(V) \alpha_3(V)], [\alpha_1(V)]_3$		[19]
		XI	$[\alpha_1(XI) \alpha_2(XI) \alpha_3(XI)]$		[20]
		XXIV	$[\alpha_1(XXIV)]_3$		[21]
		XXVII	$[\alpha_1(XXVII)]_3$		[22]
FACIT collagen	<ul style="list-style-type: none"> • To serve as a molecular bridge to enhance the organization and stability of ECM • To facilitate the adhesion and interactions between collagen fibers and ECM • To modulate the surface properties of the fibrillar collagens 	IX	$[\alpha_1(IX) \alpha_2(IX) \alpha_3(IX)]$	Collagen fiber surface	[23]
		XII	$[\alpha_1(XII)]_3$		[24]
		XVI	$[\alpha_1(XVI)]_3$		[25]
		XIV	$[\alpha_1(XIV)]_3$		[24]
		XIX	$[\alpha_1(XIX)]_3$		[26]
		XX	$[\alpha_1(XX)]_3$		[27]
		XXI	$[\alpha_1(XXI)]_3$		[28]
		XXII	$[\alpha_1(XXII)]_3$		[29]
Microfibrillar collagen	To interact with fibrils and cells	VI	$[\alpha_1(VI) \alpha_2(VI) \alpha_3(VI)]$	Independent microfibrillar network	[15]
Short chains collagen	To involve in the calcification process in the lower hypertrophic zone	VIII	$[\alpha_1(VIII)]_2 \alpha_2(VIII)$	Hexagonal meshwork	[30, 31]
		X	$[\alpha_1(X)]_3$		[32, 33]
Transmembrane collagen	To involve in cell adhesion and migration, hemostasis, and immune regulation	XIII	$[\alpha_1(XIII)]_3$	Adhere to the basement membrane	[34]
		XVII	$[\alpha_1(XVII)]_3$		[16]
		XXIII	$[\alpha_1(XXIII)]_3$		[35]
		XXV	$[\alpha_1(XXV)]_3$		[36]
Basement membrane collagen	To impart tensile strength to the basement membrane and anchor to the laminin network	IV	$[\alpha_1(IV)]_2 \alpha_2(IV); \alpha_3(IV), \alpha_4(IV), \alpha_5(IV), \alpha_6(IV)$	Anti-parallel tetramers	[37]
		XVIII	$[\alpha_1(XVIII)]_3$		[38]
Anchoring collagen	To ensure the integrity and stability of the basement membrane area	VII	$[\alpha_1(VII)]_3$	Anti-parallel dimers	[39]

playing an essential role in complex tissue formation and supporting biological activities *in vivo* [41]. It is abundant in vertebrate tissues such as skin, ligament, bone, cornea, and tendon [42]. Type I collagen fibrils can be self-assembled into microfibrils of varying sizes and morphology and can be modified chemically to incorporate into type III collagen for vocal repair [18] or type V collagen to promote the biomechanical performance of regenerated bone tissue [19]. Its excellent biodegradability, histocompatibility, and hemostasis make it an ideal biomaterial for tissue engineering. However, it is important to consider the various sources of collagen available and religious beliefs in specific areas of industrial production.

2.2 Hierarchical structure of type I collagen

In relation to the complete quaternary structure of collagen (Fig. 1), the primary structure consists of the Glycine-X-Y tripeptide sequences, where each triplet residue

contains glycine, while the X and Y sites are occupied by proline and 4-hydroxyproline (approximately 20%), respectively. Notably, collagen uniquely contains hydroxylysine and hydroxyproline, of which the latter occupies more than 50% compared to other amino acids in collagen. The secondary structure adopts an α -helix shape due to the steric repulsion effect between proline and hydroxyproline residues in the X and Y positions, respectively. The tertiary structure is referred to as tropocollagen and has a relative molecular weight of approximately 300 kDa [43]. The quaternary structure refers to the supramolecular aggregation based on microfibrils and fibers. Tropocollagen is combined terminal to terminal and arranged in a parallel and ordered mode accompanying the quarter staggered arrangement between head and tail with a *D*-periodic banding space (around 67 nm) due to the electrostatic and hydrophobic interactions.

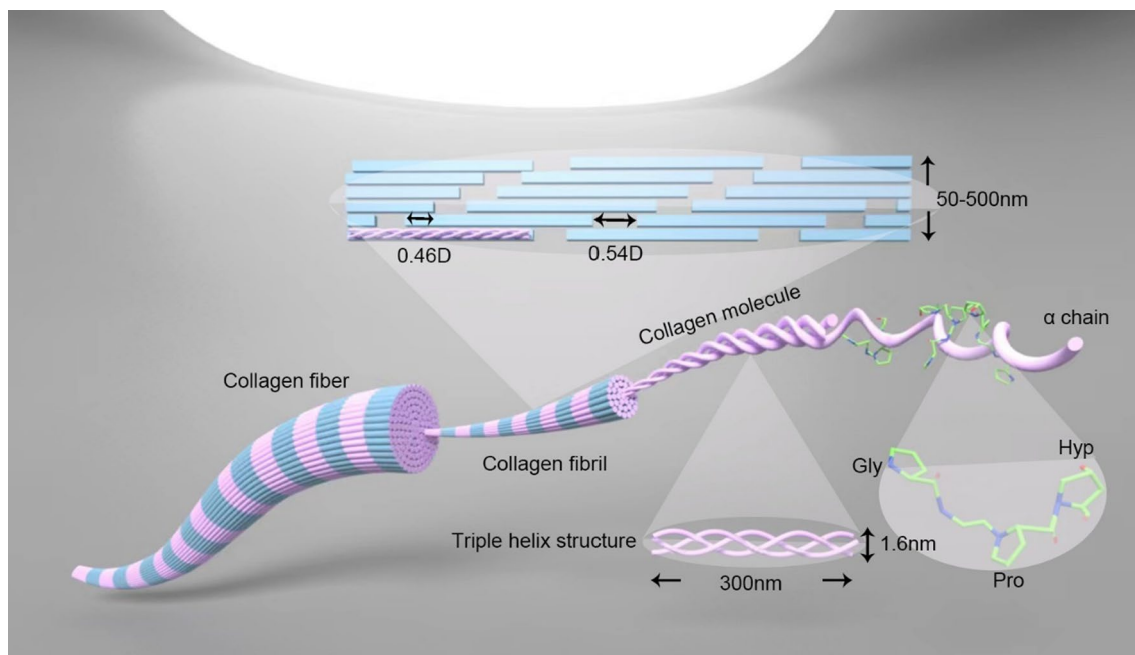


Fig. 1 Multi-hierarchical structure of type I collagen

The primary structure of collagen is composed of linear sequences of interconnected polypeptide bonds, which are mainly influenced by the amount, type, combination, and order of amino acids. The unique composition and arrangement of proteins give rise to the specific spatial structure and multi-hierarchical organization of collagen. Throughout the chain, the amino acids are arranged in repeated periodic patterns such as (Gly-X-Y), including glycine, proline, and hydroxyproline. Hydroxyproline, which is formed from proline through hydroxylation by hydroxylase, provides binding sites for water molecules in polypeptide bonds, promoting hydrogen bond formation and stabilization of the triple helical domain [44]. Similarly, hydroxylysine, another characteristic amino acid in collagen, is derived from lysine through hydroxylation. The hydrogen bond, van der Waals force, and the covalent bond can be established between two typical amino acids in the collagenous region, which contribute to the structural stability and self-aggregation of collagen in the ECM [6].

The secondary structure of collagen pertains to the ordered spatial arrangement achieved by adjacent amino acids in the polypeptide chains, which is attributed to the regulated folding of the chains. The left-handed α -helix structure is crucially important in this β -fold structure. This β -fold structure is primarily established through electrostatic repulsion between proline and hydroxyproline, stabilized by the establishment of hydrogen bonds between adjacent amino acid residues. These residues

are extended in the outward direction, thereby forming multiple hydrogen bonds in the helical domain, which provides higher conformational stability [45]. The folding of natural proteins into specific aggregated fibers, including β -sheet, β -fold, and α -helices, is largely determined by their sequence of amino acids and the construction of their polypeptides. The α -helix structure, for instance, can be transformed into disc coils in the secondary structure of collagen [44].

The tertiary structure of collagen is transformed by the further wrapping and folding function of secondary bonds between polypeptide chains based on the secondary structure. The triple helical domain is prevalently found in almost all constructions, composed essentially of three cross-linked left-handed helical polypeptide chains to synthesize a right-handed triple helix or superhelix, known as tropocollagen. Tropocollagen has a length of approximately 300 nm, a diameter of 1.5 nm, and a weight of nearly 300 kDa [43]. The collagen molecules are unable to coil around in the standard α -helix pattern due to the significant presence of proline and hydroxyproline in the molecules. Furthermore, the interleaved arrangement of the three α -peptide chains places the Gly, X, and Y residues at the same level, facilitating the formation of strong hydrogen bonds between the N-H group in Gly residues and the hydroxyl group on the neighboring X residue for enhanced molecular stabilization [45].

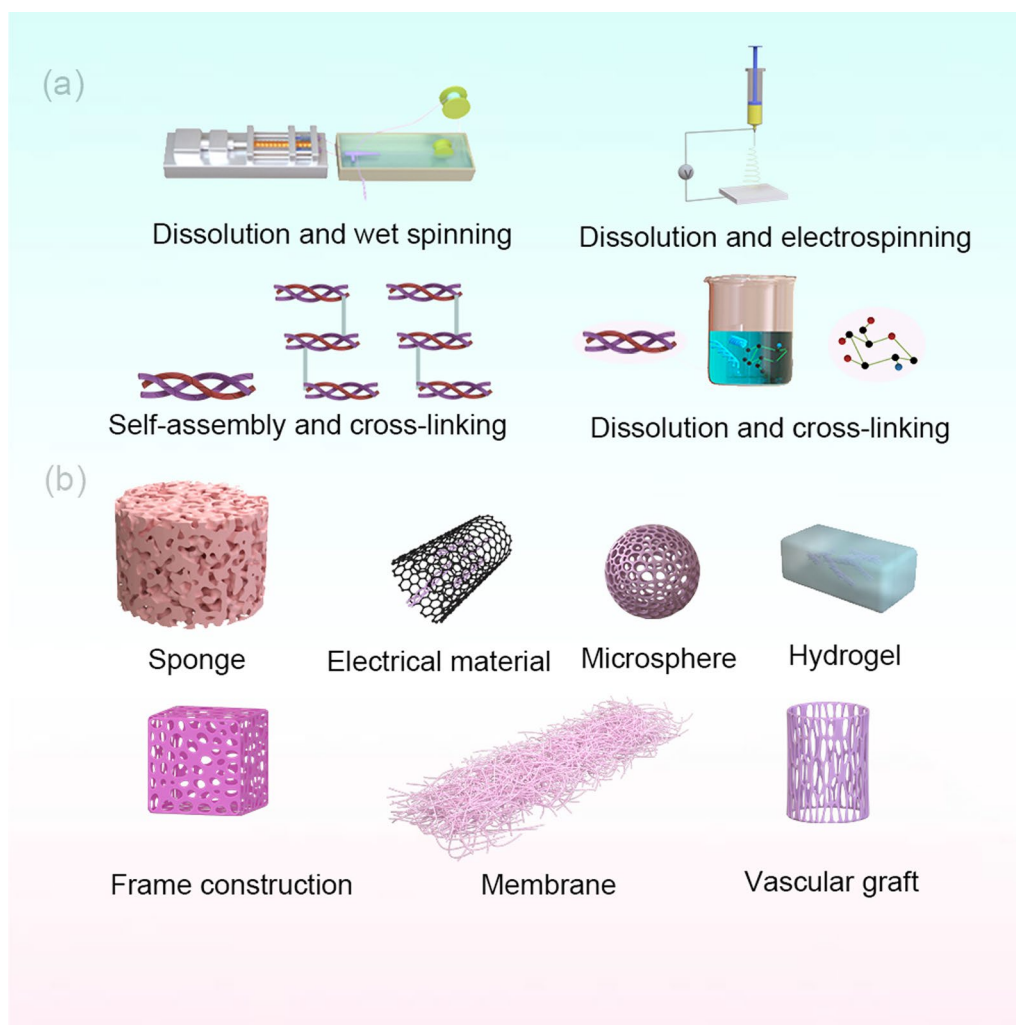


Fig. 2 **a** The main processing approaches and **b** applicable forms of collagen

Tropocollagen is arranged in staggered quarter-periods, running in parallel and cross-linked via covalent bonds to synthesize anchored collagen microfibrils with D-cycle band spaces of approximately 64 or 67 nm, further leading to the formation of collagen fibers. These hydrophobic fibers are altered through intramolecular or intermolecular cross-linking effects, and collagen is unable to be bound by disulfide bonds like keratin due to the absence of cysteine [46]. In addition, the alternating occurrence of amino acids in the α chain between polar and non-polar regions due to their electrostatic gravitational force results in a void region on the same axis or adjacent axis between two collagen molecules [42]. Collagen must not be interpreted as mere proteins containing numerous fibrils. In skin, protofibrils exhibit a distinctive trimeric structure with a uniform diameter (~ 100 nm),

whereas corneal tissue reveals a narrower diameter (~ 40 nm) in a laminar structure. Meanwhile, microfibrils can be axially stretched to further aggregate into collagen fibrils and finally twisted into bundles with a wide range of diameters (50–500 nm) once oriented in the same direction in tendon tissues [47]. Collagen composed in different structures consistently exhibits different characteristics, particularly regarding thermal stability and mechanical strength, which paves the way for a wider range of potential applications based on collagen biomaterials [48]. Aside from the degraded primary structure of collagen when heated, there are changes in state and color. Nonetheless, the thermal degradation of collagen under appropriate conditions is a potential approach to treating organic solid wastes [49].

3 Powerful biological performances of collagen

To achieve its potent biological performances, collagen has been processed into various forms to promote cell growth, proliferation, differentiation, and activity [50]. Significantly, the combination of collagen with other substances results in the formation of network structures in the ECM, creating an optimal microenvironment for cell proliferation. Epithelial and endothelial cells can attach to the collagen surface or within the ECM with ease [51]. Most research on collagen in biomaterials centers on its antioxidant properties, which are attributed to repeated Gly-Pro-Hyp sequences and amino acid composition. Additionally, collagen exhibits a plethora of other biological functions, including inhibitory, antitumor, and anti-freeze activities, and it plays a role in regulating biological processes and promoting tissue regeneration.

3.1 Antioxidant ability

Collagen from different sources exhibits extensive antioxidant capacity. For instance, collagen extracted from the skin of Crimson Snapper (*Lutjanus erythropterus*) and Silver Pomfret (*Pampus argenteus*) both demonstrate scavenging activity towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide radical along with ferric-reducing performance [52]. The antioxidant properties of collagen are mainly associated with amino sequences and hydrophobic amino acids, such as the antioxidant abilities linked with the Gly-X-Y repeat sequence, glycine, and proline from swim bladder collagen [53]. Scavenging DPPH generally involves synergistic interactions of antioxidant mechanisms for lipid peroxidation inhibition, and/or chelation of iron or copper ions, for instance, collagen shows scavenging free radicals and metal chelating properties by suppressing peroxide formation and lipid oxidation. Furthermore, metal ions in chelates could contribute to collagen antioxidant activity [54].

3.2 Inhibitory activity

The inhibition of several enzymes by collagen may have potential therapeutic benefits. In the case of hypertension treatment, the inhibition of angiotensin I-converting enzyme (ACE-I) is desirable. Collagen has been shown to effectively suppress ACE-I activity, which contrasts with the known side effects of synthetic ACE-I inhibitors such as cough, taste disturbances, skin rashes, and angioneurotic edema [55]. Specific binding between ACE-I and three specific amino acid residues at the C-terminal of collagen is responsible for its inhibitory effect; for instance, Pro residues at the C-terminal of collagen molecules from tilapia skin were found to contribute to ACE-I inhibitory activity [56].

In addition to the inhibition of ACE-I, the inhibition of dipeptidyl peptidase-IV (DPP-IV) can lead to a reduction in blood glucose levels. Fish skin-derived collagen hydrolysates displayed potent DPP-IV inhibitory activity, which was attributed to the length and the second amino acid of the N-terminal [57].

3.3 Antitumor activity

Tumors are an abnormal accumulation of cells forming masses. Currently, cancer treatment is ineffective and costly. Research has shown that the $\alpha3(IV)NC1$ domain has antitumor properties that could inhibit the growth of human renal cell carcinoma and prostate carcinoma in mouse xenograft models [58]. It is viable to prevent cancer by means of taking anticancer agents to inhibit the development, progression, and metastasis of cancer cells. Thus, taking fish-derived collagen may be the potential antitumor treatment because the low molecular weight of collagen peptides exhibit antimicrobial property. Apart from the direct inhibition of development, collagen can be prepared into numerous formats to load other anticancer molecules to indirectly achieve aims.

3.4 Anti-freeze activity

Collagen molecules with anti-freeze properties can enhance the storage of blood and tissue, as well as protect cell membranes to preserve various biological activities. These molecules exhibit anti-freeze abilities by controlling the growth of ice, transforming their conformation dynamically to overcome steric hindrances. Recently, scientists have developed a conductive hydrogel based on collagen that can withstand extremely low temperatures ($-60\text{ }^{\circ}\text{C}$) and is also biocompatible [59]. This hydrogel could accurately monitor the movements of the human body, showcasing its potential as an innovative biomaterial for creating biomimetic e-skins.

3.5 Regulating biological activities

Collagen has the capability to engage in a multitude of biological activities via interaction with various factors, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) in the body. Given its high binding affinity to growth factors [60], collagen was chosen to enhance therapeutic efficacy by directing VEGF localization to injured sites. Collagen formation is also regulated by distinct factors. Connective tissue growth factor may secrete the cloned *ctgf* gene to a cell culture medium and promote type I collagen expression in animals [61]. The activation of YAP by type I collagen could inhibit adipogenic differentiation, accelerating glycolipid metabolism and leading to the reduction of free fatty acid release and intracellular lipid accumulation.

This mechanism could be employed to target weight control [62]. For tissue repairing, the musculoskeletal biomaterials require osteoinductivity, environmental sustainability, and economy. Isolated collagen obtained from fish has been found to retain the immunomodulatory capacity of bone marrow mesenchymal stromal cells (BMSCs) [63]. Ensuring environmental sustainability and cost-effectiveness is crucial in the development of such materials too.

3.6 Involving the tissue recovery

Collagen could serve as a viable treatment option for a wide range of complex tissue damage situations. In cases of early osteoarthritis, the overproduction of type I collagen could lead to the degeneration of collagen II fibers and cartilage damage over time [64]. To address severe traumatic brain injuries, a type I collagen-based hydrogel mixed with sodium alginate and stromal cell-derived factor-1 could act as a carrier for BMSCs to mitigate motor and cognitive dysfunction. Owing to the high biocompatibility, the as-prepared scaffold reduced brain lesions and neuronal cell death with mitigated neuroinflammation [65]. Thanks to its high biocompatibility, the resulting scaffold can reduce brain lesions and neuronal cell death, while also mitigating neuroinflammation. Derived from biological tissues, collagen can play a crucial role in specific areas of the human body, such as forming the basic mineralized structure in bone tissue to support its mechanical strength [66]. During wound healing, collagen I and collagen III could serve as effective cellular scaffolds, facilitating the formation of new blood vessels and epithelial coverage [67]. Additionally, the amino groups in the lysine side chain of collagen are associated with VEGF and PDGF, endowing it with platelet-activating and coagulating properties similar to those of natural polymer hemostatic sponges [13]. Composite collagen sponges might have better mechanical strength, faster shape recovery, superior coagulation effects, and lower blood loss than their natural polymer counterparts [68].

4 Extraction and processing of collagen

To achieve optimal biomedical applications, it is essential to obtain pure collagen from biological tissues through effective processing techniques. Through thorough pretreatment, undesired impurities can be removed, resulting in highly purified collagen extractable with various approaches, as outlined in Table 2. In addition, the advantages and disadvantages of the different extraction methods are shown in Table 3. Following extraction, collagen can undergo different processing methods such as dissolution, self-assembly, and cross-linking to enhance its functionality (Fig. 2a). This section focuses mainly on

collagen extraction and processing methods for biomaterial applications.

4.1 Extraction of collagen

Under acidic conditions, fibrous collagen undergoes swelling and dissolution. The breaking of salt and Schiff bonds between collagen molecules allows more extraction of acid-soluble collagen (ASC) with a maintained triple helical structure (ASC) [56]. ASC can be extracted using organic or inorganic acids, with acetic acid extraction exhibiting higher yield and viscoelasticity than hydrochloric acid extraction [69]. Currently, fish-derived collagen is showing more promise than mammalian collagen. The isolated ASC from fish waste that can be blended with chitosan to create membranes with broad antimicrobial properties [70]. During the extraction of whole collagen, irreversible structural changes in the proteins should also be noted. Alkaline solutions combined with enzymes are found to produce higher yields for collagen extraction. Tang et al. used alkaline protease to further cross-link pure collagen fibers obtained from alkaline-pretreated bovine hides, which were then woven into material [71]. Alkaline pretreatment can also contribute to maintaining structural integrity and preventing ASC loss. For instance, the elimination of Nile tilapia skin resulted in the highest removal of non-collagenic protein at 24.3% [72]. Furthermore, alkaline pretreatment during collagen extraction from sturgeon showed sufficient structural stability in isolated type I and II collagens, although the fibril-forming speed of type I collagen was slower than the other [73].

Typically, collagen obtained through the use of salts is known as salt-soluble collagen (SSC) [56]. However, the extraction yield is limited due to collagen's poor solubility in low concentrations. In the extraction of SSC from porcine skin [74], differences were observed in protein degradation, emulsifying properties, and gel strength when using wet- and dry-salting methods, both of which showed augmented extraction efficiency. However, acid treatment extraction demonstrated better results compared to the use of salt.

Enzymes are utilized to facilitate collagen dissolution in acidic conditions for rapid hydrolysis without causing pollution. This enzymatic extraction method possesses unparalleled advantages in extraction efficiency and is generally referred to as pepsin-soluble collagen (PSC). While there was a significant difference in collagen yield between PSC and ASC [75], there were no differences observed in the amino acid composition and physicochemical characteristics [76]. Following enzymatic extraction, collagen retains a reliable triple helical structure with reduced antigenicity, making it a valuable resource for the healthcare industry. This has been

Table 2 Collagen extraction variance from different sources

Sources	Collagen type	Tissue	Methods	Extraction agents	Time (h)	Yield (%)	Ref.	
Sharpnose stingray	Type I	Skin	UASC	Acetic	1	11.49	[69]	
				HCl		3.5		
			UASC	Acetic and pepsin		42.34		
Black ruff	Type I	Skin	UPSC		72	61.50	[70]	
			ASC	Lactic		45.0		
				Formic		32.0		
				Tartaric		31.0		
				Citric		31.0		
	Acetic	25.0						
Nile tilapia	Type I	Skin	ASC	Acetic	5	21.4	[72]	
Bester sturgeon	Type I	Skin	PSC	HCl and porcine pepsin	48	63.9	[73]	
	Type II	Notochord				35.5		
Pork	Type I	Skin	SSCD	Wet-salting	2	58.29	[74]	
				Dry-salting		50.95		
Golden carp	Type I	Skin	UASC	Acetic	48	81.53	[76]	
			ASC			51.90		
			UPSC	Acetic and porcine pepsin		94.88		
			PSC			79.27		
Asian bullfrog	Type I	Skin	ASC	Acetic	48	8.49	[77]	
			PSC	Acetic and porcine pepsin		28.3		
Lamb	Type I	Feet	UPSC	Lactic, pepsin	7	-	[78]	
Yellowfin tuna	Type I	Skin	PSC	Acetic, pepsin	48	23.5	[79]	
			UPSC			23.84		
			ASC	Acetic		21.49		
			UASC			57.06		
Bovine	Type I	Bone	ASC	Acetic	72	6.13	[80]	
			PSC	Acetic, pepsin		10.59		
			ESC	Acetic, PsaAPA		12.18		
Starfish	Type I	Achilles tendons	UPSC	Pepsin	72	72.0	[83]	
		Bone fragments	PSC	Acetic, pepsin	48	4.01	[81]	
			HASC	Acetic		3.34		
Jellyfish	Type III	Bell	ASC	Acetic	144	~5	[85]	
		Oral arm				~7		
		Bell	PSC	Acetic, pepsin		48		~18
		Oral arm						~20
		Bell	UASC	Acetic		-		37.08
		Oral arm						40.20

ASC: Acid-soluble collagen; UASC: Ultrasound acid-soluble collagen; PSC: Pepsin-soluble collagen; UPSC: Ultrasound pepsin-soluble collagen; SSCD: Salt-soluble collagen derivatives; SSC: Salt-soluble collagen; ESC: Enzyme soluble collagen; PsaAPA: An aspartic protease from *Penicillium* sp. XT7; HASC: Homogenization acid solubilized collagen

confirmed in various sources, including Asian bullfrog skin [77], lamb feet [78], and rainbow trout [79]. Furthermore, other enzymes, such as aspartic protease, have also been shown to potentially aid in collagen extraction [80].

Ultrasonication is a rapid and dependable method of collagen digestion and extraction. By utilizing assisted sonication, intertwined collagen fibrils can be effectively separated, leading to reduced extraction cycles [76] and

enhanced collagen extraction efficiency, structural stability, denaturation temperature (T_d), solubility, viscosity, rheology, water-holding capacity, and emulsifying properties [81–84]. Furthermore, through the use of physical treatment, collagen extraction efficiency is substantially improved, resulting in reinforced solubility and uniformity [85].

Table 3 The advantages and disadvantages of the different extraction methods

Methods	Advantages	Disadvantages	Ref.
ASC	<ul style="list-style-type: none"> • No damage to amino acid composition and physicochemical features • Relatively high yield of collagen extraction 	<ul style="list-style-type: none"> • Long extraction period • Excessive chemical and processing time in the pretreatment 	[69, 70]
PSC	<ul style="list-style-type: none"> • Maintained the triple helical structure • No damage to amino acid composition and physicochemical features • Reduced antigenicity • High yield of collagen extraction • Easily combined with other methods 	<ul style="list-style-type: none"> • Long extraction period • Excessive chemical and processing time in the pretreatment 	[75, 78]
SSC	<ul style="list-style-type: none"> • Preserved thermal stability and fibril formation capacity of collagens 	<ul style="list-style-type: none"> • Limited extraction yield when in low collagen concentrations • Resource demanding salting-out • Too long extraction cycles • Lower purity 	[56, 84]
UASC	<ul style="list-style-type: none"> • Improved outcomes atop ASC • Reduced chemical and processing time in the pretreatment 	<ul style="list-style-type: none"> • Complicated steps 	[76]
UPSC	<ul style="list-style-type: none"> • Reduced extraction cycles • Improved outcomes atop PSC • Reduced chemical and processing time in the pretreatment 	<ul style="list-style-type: none"> • Complicated steps 	[76]
HASC	<ul style="list-style-type: none"> • Enhanced solubility and uniformity of collagen in solution • Reduced chemical and processing time in the pretreatment 	<ul style="list-style-type: none"> • Complicated steps 	[85]

4.2 Processing of collagen

In the context of biomaterial applications, collagen extraction from body tissues should be processed. This section delineates the primary methods of collagen processing and associated considerations. Figure 2 elucidates the principal processing techniques and applicable collagen formats.

4.2.1 Dissolution

Dissolution is a commonly employed method for the facile processing of collagen in order to fabricate novel hybrid biomaterials (Fig. 2a). In a non-neutral solution, collagen charges will migrate toward the corresponding electrodes unless they are at their isoelectric point [86]. The colloidal collagen particles do not form precipitation in water due to the effect of the hydration layer and repulsive potential force [87]. In alkaline conditions, the secondary bonds of collagen break down and eventually lead to the destruction of the multi-hierarchical structure of collagen proteins [88].

Electrospinning technology can serve as a subsequent method for the dissolution and processing of collagen into various formats (Fig. 2a). To overcome challenges in electrospinning pure collagen, a variety of materials have been employed, such as volatile organic solvents to prevent protein denaturation (for instance, mixtures of ethanol and water), natural antimicrobial substances for use in biomaterials (such as hyaluronan, chitosan, and hydroxyapatite), and synthetic polymers for producing reinforced collagen-containing hybrid fibers (including polycaprolactone, polylactide-polyglycolide,

polyurethanes, and blends of these materials) [89]. The resulting collagen nanofibers generated through electrospinning can serve as natural tissues to support various bio-behaviors. For example, hybrid collagen nanofibers based on polycaprolactone/collagen/heparin can replicate vascular biomechanical performances and enhance cell proliferation *in vitro* [90]. As a viscoelastic fiber with notable stress relaxation, collagen's mechanical strength is mostly determined by its chemical composition, cross-linking, and helical structure [91]. Compared to synthetic biomaterials, the fibrillar architecture produced through electrospinning is more alluring for cell adhesion and proliferation. However, electrospun collagen nanofibers are unable to exhibit the varied functionalities of native collagen [92]. Therefore, current research should not only focus on electrospinning collagen from relatively benign solvents but also on preserving the triple helical structure of native collagen for possible diverse functions.

When collagen fibers are dissolved in an acidic solution, they exhibit swell ability from two distinct perspectives [93]. One perspective attribute the swelling mechanism of collagen fibrils to maintaining thermodynamic equilibrium, generated by osmotic pressure that drives water molecules away from collagen. Another view ascribes the propensity of collagen fibrils to swell to electrostatic repulsion, possibly associated with polypeptide chain charge. Tang and Pei et al. conducted a series of investigations on dissolving collagen aggregates from bio-wastes or natural tissues to building blocks for isolation in aqueous solutions [94, 95]. The ability of collagen fibers to dissolve in various solvents has been well-established

[96], including collagen dissolving in NaBr aqueous solutions without excessive degradation [97]. The evolutionary mechanism of bovine collagen hiding conformation in Na_2S solution was clarified, whereby its capacity for hydrolysis follows the order $\text{NaOH} < \text{NaHS} < \text{Na}_2\text{S}$ under the same conditions. Researchers further developed a green, high-efficiency processing approach for isolating collagen fiber bundles after pre-swelling bovine hides [71], which was comparable to conventional NaOH pre-swelling in reducing pollutant load. Specifically, treated pollutant loads result in 58%, 76.75%, 76.03%, and 61.07% reduction in chemical oxygen demand, ammonia nitrogen, total solids, and discharged wastewater, respectively. After exfoliating collagen fibers in a NaOH/urea aqueous system from the bovine achilles tendon [2], the self-assembly behavior of isolated collagen fiber was studied by varying pH, ionic strength, temperature, and solvent [88]. Furthermore, collagen fibers were fabricated via wet spinning, exhibiting maximum tensile strength and Young's modulus of 9.98 cN/tex ($219.29 \pm 22.92 \text{ MPa}$) and $43.95 \pm 1.11 \text{ cN/tex}$ ($966.20 \pm 24.30 \text{ MPa}$), respectively, providing novel possibilities for collagen applications in biomedical fields [91].

4.2.2 Self-assembly

The self-assembly behavior of collagen enriches the biological tissues and hierarchical construction. Currently, collagen fibers are able to self-assemble into highly organized micro construction under distinct mechanisms including template self-assembly [98], in situ self-assembly [99], oriented self-assembly [100], and induced self-assembly [101]. The ideal processing of self-assembly can enlarge the application range of collagen-based biomaterials and the factors that influence the processing of self-assembled collagen are summarized in Table 4.

Previous studies have shown that other factors, such as the location and number of metal chelation sites, may also play a critical role in modulating the morphology of the peptide–metal assemblies. Su et al. attempted to induce alginate into the collagen assembly, leading to the formation of 3D mineralized construction with reinforced mechanical strength [102].

As a thermally-sensitive protein, collagen undergoes a transformation from its triple helical structure to disordered coils when heated above T_d [103]. Heating up within the T_d may accelerate the self-assembly process by producing finer fibers. Increased concentration resulted in visible aggregation and intermolecular interaction of collagen, promoting self-assembly formation. However, the required effective concentration for self-assembly varies depending on the source of collagen. For example, concentrations over 0.3 mg/mL are necessary for skate and sturgeon cartilage collagen [105], while walleye

pollock skin collagen requires 0.6 mg/mL [104]. The ionizable groups could regulate electrostatic interactions when treated under different pH conditions, thus affecting the processing of assembled collagen. The effect of ionic strength on collagen self-assembly is complex. To some extent, the self-aggregation rate is accelerated with continuously enhanced ionic strength, e.g., promoted collagen self-assembly with NaCl concentration from 0 to 120 mmol/L [105]. However, the opposite view emphasized that the self-assembly of type I collagen was restricted under high concentrations of NaCl solution [104] and even accelerated self-assembly with a decrease in ionic strength [52]. Contradictions can be attributed to excessive ions in the solution that increase the mutual repulsive force among collagen molecules and break the charge neutralization on collagen surfaces.

The self-assembly behavior of collagen primarily occurred in vitro. Initially, collagen fibrils exhibit a propensity to self-aggregate in a mildly neutral buffer solution [106]. Cone-shaped collagen fibrils with a diameter of approximately 20 nm and a length of approximately 5 μm tend to gather in a unidirectional manner from N- to C- terminal, ultimately enlarging into fibrils with diameters of 20–80 nm. In dilute collagen solutions, lateral aggregation among fibrils is constrained, while lengthening occurs along the axial direction. The assembly mechanism depends on the integrity of the telopeptide and the neutralization treatment of collagen. In addition, computational models using collagen monomers have aided in understanding collagen self-assembly in vitro. The Brownian motion influences the self-assembly process of collagen, which can be understood as a minimization of free energy [106]. Various models have been proposed to mimic the self-assembly behavior of collagen in vitro. For instance, the collagen monomer can be considered a flexible chain of 200 beads that exhibit attractive and repulsive effects [110]. However, the model system's relatively low scale limits its applications. To comprehend the self-assembly mechanism of supramolecular collagen, sequence data can be utilized to predict the periodic collagen structure and axial offset among molecules [111]. The extraordinary aspect ratio and wide range of lengths of collagen have resulted in a diverse range of collagen assembly models, highlighting its significant processing potential.

While the mechanisms of collagen self-assembly in vitro are clear, research on self-assembly in vivo remains a challenge. Collagen processing models in vitro can be predicted based on collagen monomers to form specific tissue with corresponding distribution in the body, due to the lack of restriction on fibril length, quantity, and orientation, such as parallel bundles in tendons [42]. Therefore, doubts remain

Table 4 Factors influencing collagen self-assembly behaviors

Factors	Phenomenon	Mechanism	Ref.
Temperature	<ul style="list-style-type: none"> • Collagen transforms into disorderly coils from the triple helical structure. • Self-assembly is associated with thermodynamic differences of collagen types in terms of secondary structure. • Temperature-induced and accelerated collagen aggregation within the Td values. 	Hydrophobic interaction	[103]
Ionic conditions	<ul style="list-style-type: none"> • Collagen self-aggregation speed is generally improved with constantly increasing ionic strength. • Na⁺ could affect and regulate the self-assembly behavior of collagen by driving fibers to form a dense meshwork structure. • Ca²⁺ hindered the rapid swelling of collagen fibrils in alkaline conditions. • The excessive ion strengths augment mutual repulsive force among collagen molecules to inhibit the faster formation of collagen fibrils. 	Electrostatic interaction	[52, 71, 104, 105]
pH	<ul style="list-style-type: none"> • Collagen fibrils demonstrated the self-aggregation propensity in neutral pH conditions and slower fibril-forming speed in alkaline conditions. • Collagen self-aggregation would be more obvious when pH is near the isoelectric point. • The formation of thick fibril-bundle structures was inhibited in alkaline conditions. 	Hydrogen bonding, hydrophobic and electrostatic interactions	[73, 106]
Concentration	<ul style="list-style-type: none"> • The higher collagen concentration leads to obvious collagen aggregation. • The lower collagen concentration results in the restricted lateral gathering among collagen fibrils while extended in the axial direction. 	Hydrogen bonding, Covalent interaction	[104–106]
Telopeptides and amino acids	<ul style="list-style-type: none"> • Telopeptides integrity determined fibril assembly route. • The arrangement order and sequences of amino acids influenced the multi-hierarchical structure of collagen. • The bond between amino acids contributes to the self-aggregation of collagen in ECM. • Amino acid residues could enhance conformation stabilization. • Glycine substitutions in collagen heterotrimers could result in the formation of disc coils from the α-helix structure. 	Hydrogen bonding, covalent interaction	[6, 106]
Hydroxyapatite	<ul style="list-style-type: none"> • The hydroxyapatite could adjust the diameter and porosity among self-assembled collagen fibrils. 	Wrapping the nucleation site groups	[107]
Cross-linking agent	<ul style="list-style-type: none"> • Glutaraldehyde facilitated a faster stable meshwork formation of collagen fibrils. • Dialdehyde starch improved the porosity of assembled collagen fibrils. • EDC-NHS promoted the collagen fibrils to form thicker bundles locally align. 	Covalent interaction	[108, 109]
Ultrasound	<ul style="list-style-type: none"> • The twisted collagen fibrils were opened. • Ultrasound accelerated the process of collagen self-assembly 	Covalent interaction	[76, 82]

regarding how collagen fibrils assemble in vivo. Regulating the genetic code of collagen may be a contributing factor. Tenascin X, encoded on fibrillar collagen, has been associated with Ehlers-Danlos syndrome [112]. The lack of tenascin X in mouse skin resulted in a 40% reduction in collagen fibrils. The role of tenascin X in collagen fibril formation may contribute to our understanding of the self-assembly of fibrils in vivo and may shed light on other similar collagen formation

disorders. Cellular activity could also influence self-assembly in vivo. For example, cells could affect pH to indirectly affect procollagen processing and thus collagen self-assembly [113]. Moreover, cells stress adjacent fibrils to reconstruct the collagen fibril network, while the deeper mechanism remains unknown. To further our understanding of the control of cells on collagen self-assembly behavior, Rutenberg et al. aimed to analyze the molecular orientation as a whole entity rather

than the arrangement of single molecules [114]. The random fiber network can be caused by cross-linking, and reconstructing into dense and parallel bundles to cope with the extracellular force. Whether macroscopic self-assembly phenomena are related to cells remains to be further investigated.

4.2.3 Cross-linking

The diverse modification of functional groups enriches collagen processing. In this section, several crosslinking agents and methods for collagen modification are discussed.

Glutaraldehyde can be used to prevent thermal or enzymatic degradation of collagen, as well as to improve the mechanical strength of collagen-based biomaterials. The ϵ -amine groups of lysine or hydroxylysine residues in collagen can be cross-linked with the aldehyde group of glutaraldehyde, and the resulting Schiff base from neutral conditions can be further processed for tissue engineering [108]. Dialdehyde starch has been used in the design of collagen-based scaffolds [115] or membranes [116] to achieve high porosity for cell proliferation. The interaction between aldehyde groups and amino groups during crosslinking has increased the feasibility of collagen links via dialdehyde starch bridges. Collagen can react with negatively charged proteins to contribute to the preservation of the native collagen structure, thus improving porosity, swelling, and cell adhesion for cell proliferation [117]. Due to their ideal biocompatibility, collagen-based biomaterials are suitable for biomedical applications. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) are commonly combined to enhance the physicochemical capabilities of collagen under specific molar ratios. EDC-NHS serves as an intrafibrillar cross-linker to drive collagen fibrils into thicker bundles in local alignment [109], promoting collagen self-assembly function on cellular signaling, as seen from EDC-NHS cross-linked localized piezoelectric response to collagen hydrogel. Genipin, characterized by antibacterial, antineoplastic, and anti-inflammatory properties, induces cross-linking reactions with improved biomechanical properties. EDC-NHS and genipin enable precise control of collagen's biochemical and mechanical performances [118]. The EDC-NHS system exhibits short-range cross-linking formed by amino and carboxyl groups, while genipin induces long-range cross-linking by nucleophilic reaction with a much higher degree of equilibrium cross-linking than the former. In as-prepared collagen-based composite hydrogels, increasing cross-linked genipin content increases the compressive stress from 0.04 to 0.84 MPa, which may be attributed to inter- and intra-molecular covalent bond interactions in covalently bonded network structures [119]. In addition to chemical

crosslinkers, natural crosslinkers such as advanced glycation end products could also induce intermolecular crosslinks by connecting free amino groups of adjacent collagen molecules [120].

Physical cross-linking involves modifying and cross-linking collagen to prevent antigenic reactions between chemical components. The dehydrothermal (DHT) approach aims to induce additional intramolecular amide bonds between collagen exposed to high temperatures under vacuum conditions to achieve cross-linking [121]. Zhang et al. developed a cross-linked porous collagen/hydroxyapatite scaffold using DHT, achieving highly effective bone repairing with adjustable porosity and swelling ratios, as well as improved mechanical performance [122]. The addition of DHT also improved the scaffold's structural stability with decreased enzyme and culture medium degradation, leading to increased cell adhesion and proliferation [123]. UV irradiation can also induce collagen cross-linking, resulting in a unique and multilayered network structure when applied at low temperatures [124]. Specifically, collagen crosslinking occurred during fiber aggregation, which may inspire the preparation of novel collagen-based biomaterials from the spontaneously assembled collagen upon UV irradiation. However, this method may also lead to collagen photodegradation due to the loosening of the triple helical conformation [125]. Similarly, gamma irradiation can be used to crosslink collagen to enhance fracture and wear resistance [126]. Freeze drying can also modulate pore structure and improve mechanical properties to meet the requirements of crosslinked collagen. Tang et al. fabricated a type I collagen-based composite scaffold with freeze-drying, enhancing ultimate tensile strength and providing a suitable environment for cell proliferation [127].

Enzymatic cross-linking involves the covalent cross-linking of specific active sites in collagen molecules through the catalysis of enzymes, primarily transglutaminase and lysyl oxidase. This method exhibits a mild reaction and no by-products when compared to physical and chemical cross-linking [128]. Transglutaminase can improve collagen structure by forming ϵ -(γ -glutamyl)-lysine covalent bonds. Sun et al. evaluated the effect of enzymatic collagen crosslinking on the porcine sclera and confirmed a significant increase in biomechanical strength. The collagen with increased bundle density exhibited an overall elastic modulus of 14.89 ± 6.05 MPa and 12.88 ± 4.29 MPa in the double-sided and single-sided collagen crosslinking groups, respectively [129]. Enzymatic crosslinking can be combined with other approaches as well. Lysyl oxidase can catalyze the oxidative deamination of ϵ -amino lysine

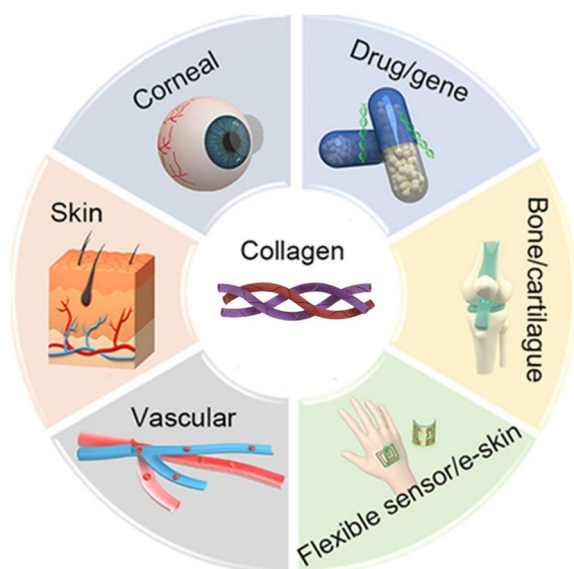


Fig. 3 Various collagen-based biomedical applications

residues to ultimately form Schiff bases, which can be utilized in conjunction with glutaraldehyde to enhance structural stability and anti-calcification properties on bioprosthetic heart valves [130].

5 Collagen-based biomedical applications

In this section, collagen-based biomedical applications are discussed in drug and gene delivery, tissue repairing, and smart-healthcare devices. The various applicable formats are shown in Fig. 3.

5.1 Drug and gene delivery

Comparable to traditional drug delivery systems *in vivo*, collagen-based biomaterials are superior in terms of bio-availability, solubility, and stability. Synthetic collagen

membranes and gels extracted from marine eel fish served as antimicrobial and antifungal carriers that demonstrated potential and broad sources for drug delivery *in vitro*, suggesting a promising outlook for biomedical application [131]. High doses or systemic administration may result in unexpected side effects. To control the release of bioactive molecules, Wang et al. fabricated a collagen and hydroxyapatite-based liposome-conjunct scaffold to better preserve and localize loaded drug molecules with the lowest release rate [132]. Similarly, the collagen-based composite membrane also demonstrated the ability to localized hydrophobic drug delivery at low doses, demonstrating the advantage of target therapy through a variety of collagen processing for drug delivery [133]. Collagen-based scaffolds can promote wound healing through long-term effects, as opposed to the short-term effects of a single clinical treatment. Cross-linked collagen scaffolds with metal nanoparticles are compatible and could effectively absorb pilocarpine hydrochloride with the sustained release for up to 13 days, thus avoiding burst release and allowing for long-term effective therapy [134]. The collagen-based biocarrier can load nanoparticles to avoid burst release for long-term effective therapy. Yao et al. fabricated a lanthanide-collagen peptide scaffold for drug delivery with metal-organic frameworks (MOFs) for anticancer molecules [135] (Fig. 4). The pH regulation of collagen's terminal amino acids and the addition of lanthanide ions acted as an external stimulus for collagen assembly, leading to controlled drug release under acidic conditions. Drug release experiments revealed that camptothecin (34.3 mg/g) and cefoperazone sodium (111.3 mg/g) were released in the majority at pH 3.0. These results suggested that hydrophilic drugs may have a greater ease of loading or entrapment within the scaffold. Likewise, Gleaton and colleagues fabricated microcages through metal

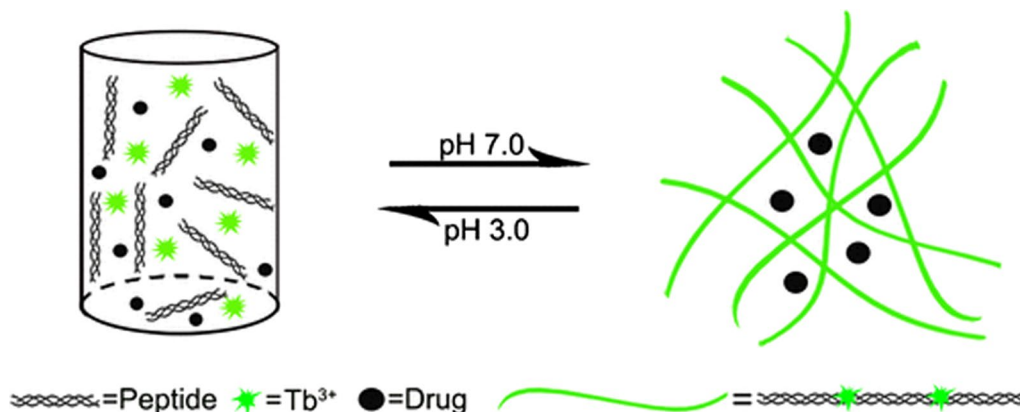


Fig. 4 The schematic of a collagen-based scaffold controlled by pH regulation. Reprinted from ref [135], copyright 2019, with permission from the American Chemical Society

promotion [136]. By leveraging the unique triple helical structure and hierarchical disks of collagen, they were able to modulate the height of the microcages by changing the polypeptide length, thereby impacting the half-life of the molecules contained within. The structure of the triple helical and microcages may be reminiscent of the construction of viruses or bacteria, which may inspire further study on the construction of microcages and enrich drug delivery forms based on collagen scaffolds.

Recently, hydrogels have gained significant interest due to their effect on water retention, structural integrity, and tissue regeneration. Shanmugapriya et al. developed a biostable composite hydrogel with improved thermal stability and non-toxicity for targeted drug delivery in cancer therapy [107]. As electronic advancements threaten human vision, an injectable hydrogel composed of alginate collagen has been synthesized for safe therapy to replace surgical treatments [137]. The biocarrier displayed sustained delivery capacity with no obvious degradation of the gel. In rats injected with intravitreal, the biocarrier demonstrated remarkable retention in photo-receptor and retinal function, as well as increased therapy efficiency with a double dose. These findings suggest that the biocarrier has a stabilizing and loading effect, making it a promising and safe approach for treating ocular diseases. Physically cross-linked collagen could deliver consistent therapeutic release while possessing biocompatible, bio-adhesive, and sprayable properties. Anderson et al. utilized a spray method to produce hydrogel for topical delivery, which could rapidly transform into a gel and adhere to the target site after shear thinning [138]. The powerful adhesion of collagen hydrogel with a controlled releasing degree makes an unparalleled advantage in terms of localized collagen-based biomaterials for targeted treatments. To achieve bone repairing, Xu et al. fabricated composite membranes consisting of polydopamine, graphene oxide, and type I collagen for coating on titanium [139]. This multi-layered collagen-based membrane could improve the biocompatibility of titanium implants by accurately controlling the loading amount and releasing ratio of bioactive components, which inspired more feasibility in clinical treatment based on the processing of collagen-based biomaterials. A collagen matrix can be a suitable carrier for solving the issues of bioavailability and stability of drug molecules. Spider silk has spurred much attention on tissue engineering due to its outstanding physical performance, biocompatibility, and bio-denaturation, however, limited in recombinant protein application. Peng et al. have induced recombinant spider silk protein in collagen matrix after modification to synthesize composite membranes [140], which enhanced the fracture stress (enhanced to 1.1- to 2.3-fold) with a more transparent appearance and maintained bioactivity.

The encapsulation also improved the cell adhesion of spider silk compared to the unloaded insoluble samples with maintained bioactivity, which provided more ideas for collagen-based composite biomaterials as drug carriers. Many pollutants are released during the production of leather. Camila et al. developed a novel approach to promote the utilization of chromium-free protein as a controlled drug carrier [141]. After recycling and purifying from industrial landfills, the maintaining and releasing abilities of silver sulfadiazine in as-prepared collagen membranes have been evaluated as well as the growth inhibition on diverse bacteria. The results indicated effective drug delivery and bacteria inhibition, which provided a new research perspective on the association between the environment and drug delivery.

In recent decades, gene-based treatment has been regarded as hopeful in cancer-inhibited therapy. RNA interference is an effective tool for gene treatment and is applied in extensive cases, particularly in cancers [142]. Collagen-based biomaterials can deal with difficulty in crossing the biological film of siRNA. The hydrogels formed by collagen *in situ* acting as Id1-targeted siRNA vehicles exhibited the availability of partial inhibition on *in vivo* gastric cancer [143]. Briefly, the addition of polyethyleneimine to collagen could promote a combination of carriers and target cells and further suppress tumor proliferation. Moreover, the type I collagen-based scaffold can be regarded as a double drug delivery system to achieve cell penetration of MicroRNA 21 for the repair of spinal cord injury [144]. Epidermal growth factor receptor (EGFR) is a vital factor to induce lung cancer. Nowadays, physical nano gold has been conjunctly utilized with collagen to improve siRNA on encapsulating EGFR siRNA for inhibiting EGFR expression and beating lung cancer [145]. As the *in vivo* model indicated, the tumor weight of mice treated by the collagen-based vehicle was reduced by 70% compared to the 30% under another carrier therapy.

In the practical application of drug and gene delivery, type I collagen, a fibrillar collagen, is commonly utilized in clinical cases. This particular collagen can be widely extracted from various marine and terrestrial animal tissues and might serve as a three-dimensional framework for tissues and organs, facilitating targeted therapy through the loading of drugs and genes.

5.2 Tissue repairing

5.2.1 Bone and cartilage repairing

Bone tissue is susceptible to injury as it is the primary load-bearing structure in the body. Polyvinylidene fluoride scaffolds have been found to exhibit a similar surface potential to cell membranes, inducing collagen fibers to become rigid under mineralization for promoting bone

tissue regeneration [146]. This approach involves altering the surface potential of polyvinylidene fluoride fibers with voltage polarities during the electrospinning process, with no additional chemical modifications needed. Another method for obtaining mineralized collagen is through the use of amorphous calcium phosphate additives [33]. Insufficient mineralized collagen might result in reduced stiffness of bone tissue, increasing the risk of bone metastasis and ultimately leading to breast cancer [147]. Collagen is capable of inducing osteogenic differentiation for bone repairing by providing a suitable microenvironment [148]. Currently, the focus of bone defect recovery is centered on promoting osteogenic differentiation via the use of mineralized collagen gels [149]. Enlightened by the pearl assembly procedure, the multi-structure of the gel with the addition of graphene oxide and hydroxyapatite was established. These porous gels are capable of promoting stem cell reproduction and osteogenic differentiation, while also providing a microenvironment for host-derived cells. Su et al. further enhanced the mechanical strength of mineralized collagen scaffolds by incorporating alginate into the collagen assembly, resulting in the formation of 3D mineralized constructs [150]. Cell experiments indicated significant proliferation of BMSCs and osteogenic differentiation in the resulting microenvironment.

Cartilage is a connective tissue situated in articulating joints, whose function is to support movement or resist compressive forces. Biomaterials derived from the cartilaginous ECM, such as type II collagen and chondroitin sulfate, have been considered to be effective in enhancing cartilage regeneration by stimulating BMSCs. However, the clinical application of chondroitin sulfate is limited due to its low stability and rapid enzymatic degradation, as opposed to collagen II. Previous studies have shown that composite collagen gels (type II: type I=1:3) are promising for use in cartilage repair due to their excellent mechanical properties [151]. Upon embedding in BMSCs to facilitate the differentiation process for repairing cartilage defects in rabbit femurs, it was observed that better recovery and more glycosaminoglycan production occurred in the composite hydrogels [152]. Tiwari et al. discussed the interaction between collagen and glycosaminoglycans using solid-state nuclear magnetic resonance spectroscopy [11], which should account for similar structural stability and other biomechanical characteristics associated with collagen biomaterials.

In recent times, bioprinting has generated a lot of interest in reproducing the structure of biological tissues. Collagen, an indispensable component of cartilage tissue, might be blended with cells encapsulated in the matrix to fabricate bioinks. Methacrylate groups are typically employed to improve the mechanical strength of

collagen-based gels printed at low concentrations while preventing cell proliferation from being limited at high concentrations [153]. The use of collagen sourced from marine organisms has shown good feasibility due to its short gelation time and few religious restrictions. For example, eel-derived collagen was fused with alginate to enhance stability and mechanical properties through bioprinting [154]. Alginate has also been observed to induce collagen II and hyaline-like cartilage production [155]. Similarly, the combination of methacrylate hyaluronic acid and type I collagen as bioink contributes to improved cell viability and adhesion [156]. In fact, 3D-printed scaffolds with type I collagen have been found to facilitate the formation of hyaline-like cartilage in vitro and in vivo [14], this collagen type displays notable printability and arrangement along the printing direction, indicating its greater feasibility in replicating the complexity of native tissues [157]. Additionally, collagen-derived gelatin can be used as bioinks for cartilage recovery, such as when bioprinted into chemically modified hydrogels to support cell adhesion and proliferation [158]. With the cost considered, type II collagen is currently restricted in usage, while it has demonstrated the ability to promote the expression of certain genes that contribute to cartilage repairing, comparable to type I collagen [159]. The high expression of collagen may also be linked to cell mobility, indicating the high stiffness of bone tissue.

5.2.2 Vascular repairing

Vascular tissue engineering has rapidly advanced to meet the increasing demands of heart and vascular diseases. Studies revealed that the arrangement of collagen fibers could modulate the vascular network formation [160], with non-chemical cross-linked collagen, demonstrating a well-matched match for vascular recovery [102]. Wheatgrass-derived growth factor and collagen deposition synergistically facilitate angiogenesis with enhanced antibacterial performance [161]. To avoid thrombi deposition and improve in-situ endothelialization and antithrombotic properties in small-diameter vascular remodeling, hyaluronic acid oligosaccharides are often combined with collagen fibers as scaffolds for vascularization through the preparation of electrospun hyaluronic acid oligosaccharides-modified collagen nanofibers [162]. Additionally, hyaluronic acid oligosaccharides can modify mineralized collagen for vascularization during bone reconstruction [163]. Metal nanoparticles can also be employed in vascular formation through crosslinks with collagen. Research suggests that cross-linked collagen with Au displays good vascular regeneration with augmented biomechanical properties and thermal stability [164]. The scaffolds based on praseodymium–cobaltite nanoparticles crosslinked with collagen have also

exhibited potential in facilitating MSCs attachment and angiogenesis [165]. The therapeutic efficacy of collagen in the human body was evaluated through the analysis of collagen-coated biosorption on textile vascular grafts as well as histological examinations of the grafts (Fig. 2b) [166]. The absence of the formation of an endothelial layer indicated a suboptimal therapeutic effect under this direct treatment. However, collagen demonstrated potential as an alternative for small-diameter hybrid vascular grafts [167].

5.2.3 Corneal repairing

Several bioengineering technologies, such as artificial stroma, recombinant collagen, crosslinking, vitrification, compressed collagen, and magnetically aligned collagen, have been applied to enhance mechanical strength and optical properties [168]. Evidently, artificial corneal require comparable characteristics to that of natural tissues. Specifically, based on the liquid crystal properties of ASC, an artificial stroma was created to promote the adhesion and proliferation of epithelial cells, thereby contributing to corneal recovery [168]. With regards to recombinant collagen, it is commonly manufactured into scaffolds with structured micro-architecture for the regeneration of corneal stroma by facilitating cellular growth and collagen deposition [169]. Common modifications to collagen are used in corneal repair, such as the application of hydroxypropyl methylcellulose to improve light transmittance and refractive indices [170], riboflavin to enhance the biochemical properties [171], and hyaluronic acid to improve biocompatibility [172]. From lower to higher vertebrates, collagen organization complexity increases, corresponding to the increase in stiffness. The collagen structure arrangement differs among corneal species [173], with examples including the “plywood” structure found in fish corneal, and a “chicken wire” pattern observed in bird corneal. Gene therapy has the potential to replace artificial corneas in the treatment of corneal scarring. For example, an inhibitor of differentiation 3 genes can prevent corneal keratocyte differentiation into myofibroblasts [174]. Based on biomechanical properties from distinct corneal sources (rabbit, chicken, bovine, and porcine), the external collagen network of the human corneal could also guide the development of corneal biomechanical models. To prevent potential infections following transplantation, sterilized radiation was utilized for collagen-based biomaterials used in corneal repair [175].

5.2.4 Wound healing

Wound repair involves restricted blood loss, secretion of inflammatory mediators, formation of new epidermal layers, and wound healing [176]. Collagen plays a crucial

part in the wound healing process by undergoing deposition and degradation, but its heightened relative molecular mass restricts its absorption into the skin.

Electrospun technology enriches the applicable forms of collagen to solve the limited absorption to the skin, as well as control the structure and biomechanical properties of processed collagen-based biomaterials. Electrospun bilayer scaffolds composed of fish-derived collagen and polycaprolactone, whose fiber diameter was related to fish-derived collagen content, could stimulate the activity of keratinocytes and human dermal fibroblasts for full-thickness wound healing [177]. Both freeze-dried and electrospun scaffolds are viable substitutes for treating massive full-thickness burns [178]. There remained no obvious difference in cell proliferation and organization between the two types of scaffolds in vitro. However, the electrospun scaffold has a higher engraftment rate compared to the freeze-dried scaffold. The electrospun scaffold presented a higher rate of engraftment than that of the freeze-dried one, while the bovine collagen still existed in the latter group after 8 weeks. The discovery inspired more consideration of maintaining collagen via electrospun. Distinct growth factors may accelerate wound healing by regulation. The heparinized collagen scaffold remained triple helical structured with amplified cell proliferation and surface adhesion, which was achieved via the Schiff base crosslink reaction [13]. After cross-linking, the shrinkage temperature, tensile strength, elongation at break, and water contact angle were enhanced to 75.6 °C, 14.62 MPa, 53.14%, and 25.1°, respectively. In vivo, composite scaffolds successfully achieved scarless wound repair by promoting the expression of growth factors and capillary angiogenesis. Currently, the combination of organic and inorganic materials provides a new perspective on wound healing. Electrospinning pads composed of native fish skin (Rohu) sourced collagen and bioactive glass can enhance neovascularization stimulation and ECM components reconstruction, emerging with potential for low-cost wound repair [102].

Conventional wound treatment often fails to achieve sustained effects. However, the incorporation of nanoparticles into collagen might enhance the mechanical performance, including tensile strength and elongation at break, while also providing a sustained healing therapy [179]. Further in vivo, evaluation showed that this approach could promote cell proliferation and migration with no toxic effects at the wound site. In particular, chronic wounds require skin regeneration rather than simple repair or closure. Compared to common wound dressings, a collagen-based scaffold loaded with polyphenols promoted more apparent epithelialization, angiogenesis, and collagen deposition [180]. Through a

synergistic effect between collagen and EGFR, new tissue forms uniformly, leading to unparalleled biological characteristics that exceed most wound dressings [181]. To avoid further injury from antibiotic and antiseptic overuse during chronic wound infection, collagenase or fibronectin can modify human AMP LL37. The resulting wound dressing exhibits sustained antibacterial activity for up to 14 days with high delivery efficiency. Recent research has found that LL37-adsorbed collagen-based wound dressings when combined with alginate, stimulate and regulate antibacterial abilities [182]. Additionally, chronic wounds may result from endogenous pathological factors, such as impaired vascularization leading to skin ulcers in diabetic patients. A jellyfish-derived collagen aqueous solution has produced a reduced healing period, lower inflammation, and less scar formation in a diabetic mouse model, indicating a rapid wound closure effect [183].

Collagen can be obtained from marine and mammalian species. The use of collagen from marine organisms has shown greater feasibility due to its short gelation time and fewer religious restrictions. However, in practical clinical treatment, porcine-derived type I collagen appears to be the first choice for implants. For bone and cartilage repairing, collagen can act directly by inducing osteogenic differentiation for bone repairing by providing a suitable microenvironment or indirectly mineralizing to enhance bone tissue stiffness. Due to cost considerations, type II collagen is currently restricted in use, but it has demonstrated the ability to promote the expression of certain genes that contribute to cartilage repairing, comparable to type I collagen. For vascular repairing, collagen fiber arrangement and collagen deposition can accelerate vascular network formation with a good match for vascular recovery. For corneal repairing, collagen certainly needs essential modifications, such as the addition of hydroxypropyl methylcellulose, riboflavin, and hyaluronic acid. From lower to higher vertebrates, collagen organization complexity increases, corresponding to an increase in stiffness. The distinct corneal sources (rabbit, chicken, bovine, and porcine) can be matched for different degrees of corneal damage repairing. For wound healing, fish-derived collagen is selected to be fabricated into various formats by electrospun technology to solve the limited absorption of skin, as well as to control the structure and biomechanical properties of processed collagen-based biomaterials. Although the deposition effect of collagen itself can promote wound healing, synergy with other materials can lead to better wound recovery.

5.3 Smart-healthcare devices

Collagen has garnered significant attention in the field of monitoring human health as a substrate for smart

healthcare devices. Given its renewable sources, collagen can also serve as a sustainable precursor for carbon materials. Collagen heteroatoms further promote the modification of hybrid collagen-based electronic biomaterials. Moreover, the multi-hierarchical architecture of collagen contributes significantly to high mass transfer efficiency [184]. In this section, the electronic applications of collagen-based biomaterials were reviewed, specifically as flexible sensors and e-skins.

In daily life, electronic devices require biocompatibility, breathability, flexibility, and portability. Collagen-based electronics demand surface functionalization to serve as substrate materials, such as allowing electronic materials to be directly imprinted on collagen for transferring electrical signals to biological surfaces. The fabrication forms and conductivity of collagen-based composite electronics were discussed. To make collagen a conductive biomaterial, Bishal et al. used atomic layer deposition to deposit a thin Pt film on collagen at 150 °C, which was more effective than the previous Pt deposition on other materials such as Nylon-6, cotton, paper, and hair [185]. Prior to Pt deposition, TiO₂ was deposited on the collagen surface to enhance Pt membrane conductivity. Electrical tests indicated a Pt thin film of around 27.8 ± 1.4 nm on collagen with a resistivity of 295 ± 30 $\mu\Omega$ cm. This flexible conductive biomaterial shows potential for use in implantable biosensors to avoid the need for surgical extraction of the device from the body. Salim used collagen sourced from chicken feet to prepare graphene-integrated 3D porous carbon sponges [186]. The porous structure of collagen-based carbon sponges provides rapid ion transfer pathways to achieve higher capacitance at 417 F/g, compared to other bio-composite porous carbon materials, such as human hair-derived carbon flakes (382 F/g) [187], pomelo mesocarps-derived porous carbon (245 F/g) [188] and lignin-derived porous carbon (124 F/g) [189]. While native collagen shows good potential as a flexible wearable sensor via imprinted conductive materials, it has limited electron transport capacity. Kolay et al. evaluated the electron transport capacity [190] of collagen in various forms, such as the electron transport behavior of the collagen membrane, which was similar to that of the single collagen fibril. Iron-treated collagen exhibited enhanced electron transportation capacity by up to 1000 times compared to native collagen, which inspired researchers to add other metal nanoparticles into collagen for improved electron transport.

In addition to collagen serving as a substrate, collagen-based sensors can also convert pressure stimuli into electronic signals due to their uniform multi-layered construction. Cowskin can be used as a skin-friendly substrate for electronic skin, demonstrating significant breathability with a high permeability of $3714 \text{ g m}^{-2} \text{ d}^{-1}$,

which surpassed that of the traditional polydimethylsiloxane membrane ($80 \text{ g m}^{-2} \text{ d}^{-1}$). The conformable skin sensor exhibited sensitive testing (0.144 kPa^{-1}), fast-time response (200 ms), and high operational stabilization (15 000 cycles) [12]. Given the characteristics of collagen fiber clusters in adsorbing conductive materials and providing electrical conductivity (Fig. 2b), Xie et al. developed a strain sensor based on leather through filtrating conductive aqueous dispersion for human movement monitoring [191]. The fabricated strain sensors showed excellent performance in terms of fast response time, stability, and durability attributed to the multi-architecture. Differing from most electronic skin technologies unsuitable for long-term wear, Zou et al. explored the use of leather-based electronic skin by converting external pressure stimuli into diverse signals that concur with the function of a sensory nerve in actual skin [192]. This e-skin was fabricated by filtrating acid-treated carbon nanotubes and containing them in the hierarchical structure, demonstrating a maximum sensitivity of 32.42 kPa^{-1} under 200 Pa pressure.

A variety of flexible sensors based on collagen can be produced with different material forms, such as e-skin, multilayer nanostructures, and conductive foam [13]. These can be achieved by rearranging collagen and combining it with conductive substances, such as carbon nanotubes, interdigital electrodes, silver nanowires [193], and graphene [194]. For example, the e-skin was created by embedding an elastic non-Newtonian gel into the collagen fiber sponge, followed by assembly with an interdigital electrode [195]. By imitating the protective effect of natural skin, the as-prepared e-skin displayed flexibility when in a relaxed state, yet increased in rigidity when encountering shocks, with a reduced peak force of 76.5% and an extended buffer time of 455%. This addresses the limitation of conventional e-skins, which struggle to imitate both the protecting and sensing capabilities of natural skin. A multi-functional sensor was also developed using collagen obtained from Cr-containing leather bio-waste as a substrate and polyaniline-acidified multi-walled carbon nanotube as the conductive substance [196] (Fig. 5). The sensor was capable of detecting compression, bending, and twisting strain, with a fast response time (110 ms), and wide response range (28 Pa–100 KPa). The fabulous moisture recognition and comfortableness of the fabricated sensors served as mimicked skins for a robot hand, detecting slight pressure and humidity variations. Similarly, a flexible pressure sensor was constructed with recycled collagen membranes derived from leather bio-waste as the substrate, coated with silver nanowires and an interdigital electrode, respectively [193]. The sensor demonstrated a short response time of 349 ms and relaxation time of 147 ms

under 636 Pa pressure with a sensitivity of 13.33 KPa^{-1} and 1.27 KPa^{-1} in a wide dynamic range of $64 \sim 1909 \text{ Pa}$ and $2545 \sim 6364 \text{ Pa}$, respectively, proving the potential of collagen-based biomaterials in pressure recognition and human-machine interaction.

However, the slight disparities between sensors and the human body require additional development for their use as electronic skins. Presently, a deformable piezoresistive sensor has been created using collagen fibers sourced from tannery waste, forming a conductive foam with a cross-scale multilayer structure [13]. Through its multi-layered construction, deformations spanning from the nanoscale to the microscale were accurately detected following external stress stimuli. The fabricated sensor could detect a range of human body movements, boasting a foam structure with a porosity of 92.42% and water vapor permeability of $90.76 \text{ g mm m}^{-2} \text{ h}^{-1} \text{ kPa}^{-1}$, thereby providing innovative insights for the fabrication of piezoresistive sensors with enhanced comfort and sensory properties.

A recently developed multifunctional and flexible sensor, inspired by natural hair squama, was created using a strategy involving the construction of a multilayer structure [194]. The sensor was piezoresistive and comprised of a graphene/leather-based composite fabricated using oil-tanned leather with no chromium at all (Fig. 6). Additionally, the scalable adsorption-assisted coating further enhanced the sensor's efficiency. The conductive oil-tanned leather was not only eco-friendly but also demonstrated impressive conductivity at $80 \text{ k}\Omega \cdot \text{sq}^{-1}$ and high sensitivity at 28.96 kPa^{-1} when compressed and 52.10 kPa^{-1} when stretched. Furthermore, this sensor could monitor physiological signals of plantar pressure wirelessly, unlike expensive commercial wearable sensors that only allow wired transmission. These results demonstrate the significant potential of flexible sensors or e-skins based on collagen biomaterials and inspire a trend to select eco-friendly conductive composite materials combined with collagen materials or reusing collagen from leather waste.

6 Summary and outlook

The extraction, process, and biomedical applications of collagen have broad development prospects in the biomaterial field. Collagen is a biomaterial with various advantages over other candidates for use in biomedical applications, owing to its remarkable biocompatibility, biodegradability, and low immunogenicity. The powerful biological performances of collagen are attributed to its multi-level hierarchical structures. 29 kinds of collagen have been clarified in this superfamily and its multi-hierarchical structure has been fully investigated at a molecule level. Hence, the meso-scale structures of collagen

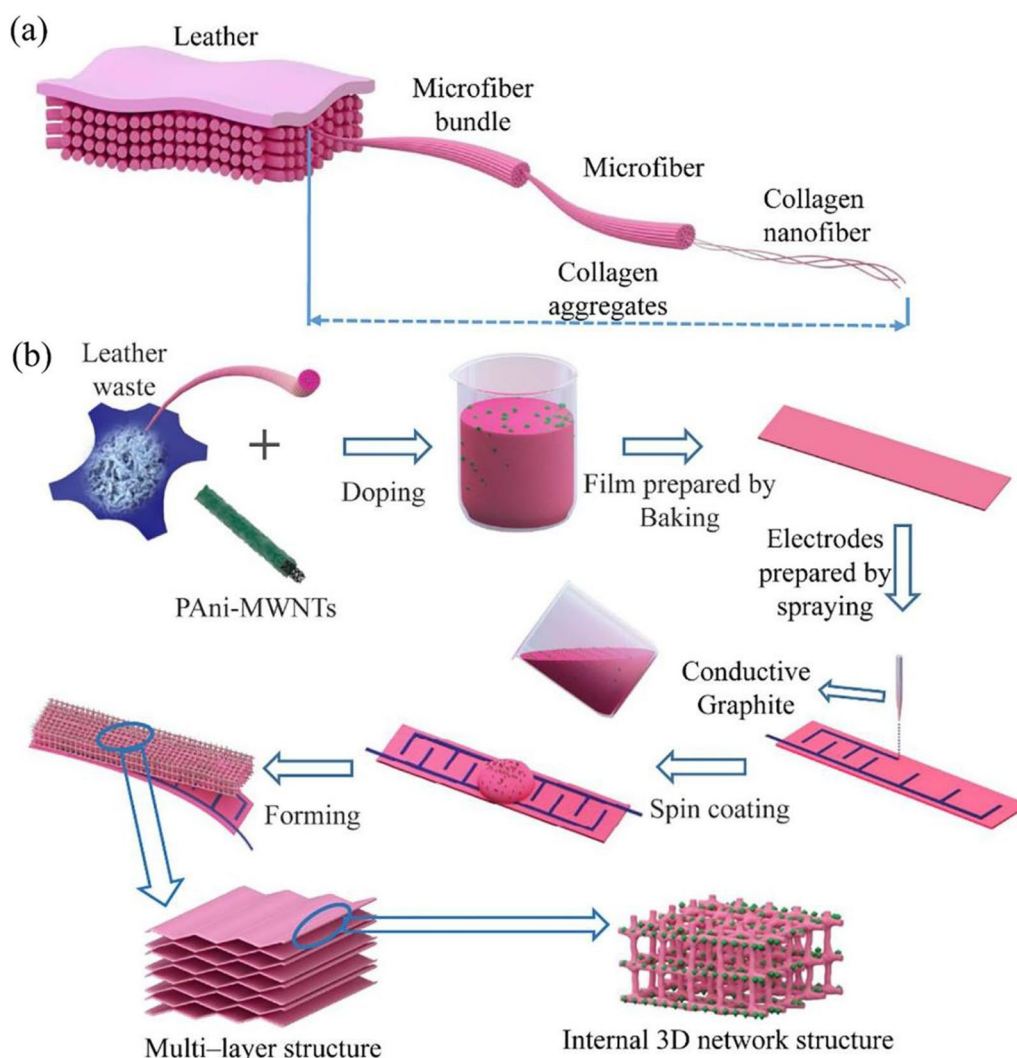


Fig. 5 Collagen aggregates in leather (a) and preparation of the sensor (b). Reprinted from ref [196], copyright 2020, with permission from Elsevier

aggregation should get more attention. Although collagen can be widely obtained from the natural aspect, the most applicable cases have been still focused on type I collagen up to now. Pure collagen can be obtained by a series of mature methods from animal tissues in terms of acid, alkali, salt, enzymes, ultrasound, and physically assisted treatments. Apparently, the PSC seems the ideal method to obtain collagen with high purification and yield after complicated pretreatment. Moreover, the combination of enzymes and physical aids can further improve extraction efficiency. After the long cycle of the extraction process, purified collagen can be processed into needed formats for further demands. Both the extraction and processing of collagen emphasize the preservation of the integrity of the triple helical structure as possible and thus the bio-activity. Although collagen exhibits powerful biological performances in antioxidant, inhibitory, antitumor, and

anti-freeze activities, as well as to play a role in regulating biological processes and promoting tissue regeneration, specific modifications are needed to enhance the therapy. The processing of collagen also involves typical strategies and methods such as dissolution, assembly, and cross-linking. Dissolution is a commonly employed method for the facile processing of collagen into desired states with other additives. The self-assembly behavior of collagen enriches the biological tissues and hierarchical construction, which is influenced by many factors including temperature, ionic conditions, pH, concentration, telopeptides and amino acids, etc. Under specific conditions, collagen can be self-assembled to form the desired Meso-level structures at an improved speed. The self-assembly behavior of collagen mainly occurred in vitro with clear mechanisms, while research on self-assembly in vivo remains a challenge. The diverse modification of

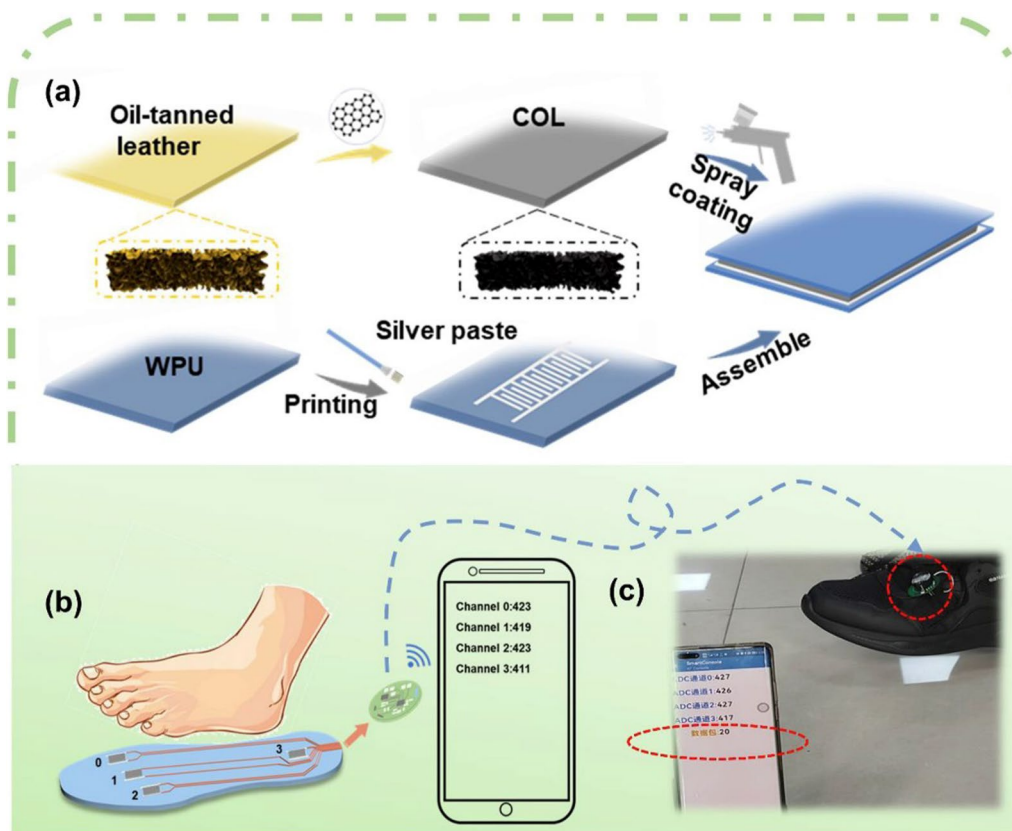


Fig. 6 **a** The fabrication of oil-tanned leather-based sensor; **b** Construction of wearable sensors system; **c** Physical diagram of the wearable sensors system. Reprinted from ref [194], copyright 2022, with permission from Elsevier

functional groups enriches collagen processing. There is an abundance of chemical crosslinking agents to facilitate the crosslinking of collagen molecules and enhance their properties. Physical cross-linking involves modifying and cross-linking collagen to prevent antigenic reactions between chemical components. Enzymatic cross-linking involves the covalent cross-linking of specific active sites in collagen molecules through the catalysis of enzymes with no by-products, compared to physical and chemical cross-linking. In biomedical applications, collagen can be fabricated into formats of fibers, membranes, hydrogels, sponges, and microspheres. The fibers can assemble into a multi-level structure for complicated demands; the membranes can protect the injured sites with a larger contact area; hydrogels are allowed to attach to target sites with augmented biocompatibility; the sponges exhibit favorable elasticity and porosity to contribute to cell proliferation and migration; the microspheres can load more drug substances for therapy. As an efficient vehicle, collagen has the significant ability to enhance the bioavailability, solubility, and stabilization of drugs and genes. Comparable to traditional drug delivery systems *in vivo*, collagen-based biomaterials are superior

in terms of bioavailability, solubility, and stability. As an ideal tissue repair biomaterial, collagen has the powerful ability to achieve repair on bone, cartilage, corneal, vascular, and wound healing. In practical clinical treatment, porcine-derived type I collagen appears to be the first choice for implants. With specific modifications, collagen-based biomaterials can achieve better tissue repairing. As a biocompatible and biodegradable biomaterial, collagen exhibits the powerful potential to be fabricated into smart-healthcare devices for intelligent health monitoring. Collagen-based electronics need to be surface functionalization to serve as substrate materials, such as allowing electronic materials to be directly imprinted on collagen for transferring electrical signals to biological surfaces. In addition to collagen serving as a substrate, collagen-based sensors can also convert pressure stimuli into electronic signals due to their uniform multi-layered construction.

Although collagen materials in recent years in the field of biomedicine have made remarkable progress, some challenges remain as follows. (1) The natural aggregation structure of collagen is destroyed in the process of dissolution and processing, which leads to

the limitation of the properties of the prepared materials. For instance, in alkaline pretreatment, the secondary bonds of collagen break down and eventually lead to the destruction of the multi-hierarchical structure of collagen proteins. Some improvements such as chemical modification, cross-linking, and blending with other components also may induce bio-safety concerns, the loss of biological properties, and compatibility issues with the blending system. For example, the collagen processing by chemical cross-linking may cause antigenic reactions between chemical components. The numerous additives may also lead to unexpected results. Also, some advantages such as biocompatibility and biodegradability of collagen should be maintained after processing. New collagen extraction and processing methods are expected to be developed. From our perspectives, more attempts at the combination of physically aided and enzymes should be made for collagen extraction. Ultrasound or high-pressure homogenization can reduce extraction cycles and enhance the solubility and uniformity of collagen in solution. To maintain the biocompatibility and biodegradability of collagen, the key is to prevent the hydrolysis of side chains. (2) The large-scale preparation of collagen materials is limited because the extraction and processing of collagen involve a series of tedious steps. In the face of the huge potential demand for biomedicine, collagen materials must be prepared efficiently and at a low cost. Relatively, collagen extracted by enzymatic method retains a reliable triple helical structure with reduced antigenicity, making it a valuable resource for the healthcare industry. However, extraction cycles seem too long due to the tedious pretreatment steps. Can the degree of purification of collagen be guaranteed in large-scale preparation? It seems that fewer additives should be utilized and more physically aided approaches should be chosen to simplify pretreatment steps. (3) The interaction mechanism between collagen materials and biological tissues needs more in-depth research, which is an interdisciplinary research field, aiming to design collagen-based biomaterials. The combined use of collagen with other biomaterials drives the question of how to select collagen types and collagen derived from different sources for different fields. The use of collagen from marine organisms has shown greater feasibility. However, porcine-derived type I collagen appears to be the first choice during clinical therapy. To design collagen-based biomaterials for specific demands, the collagen function should be known. For instance, collagen can act directly by inducing osteogenic differentiation for bone repairing by providing a suitable microenvironment. In addition, mineralized collagen can enhance bone tissue stiffness.

Thus, the collagen-based biomaterials should achieve appropriate collagen mineralization. (4) Biomedical devices require biocompatibility, breathability, flexibility, and portability. Collagen can be derived from natural renewable sources or bio-waste and be fabricated into electronic devices. The stability of collagen-based electronic devices should be further strengthened for future applications that may involve extremely harsh conditions, such as strong acidic, basic, and salt solutions and high-temperature environments. Unlike most long-term wear-resistant electronic skin technologies, leather-based electronic skin exhibits biocompatibility, breathability, flexibility, and portability. Considering collagen is the main component of leather, the design of smart health care devices based on functional leather may be a try. Briefly, it can be fabricated by embedding electrical materials in its hierarchical structure with surface coatings to prevent strong acidic, basic, and salt solutions and a high-temperature environment.

Abbreviations

ECM	Extracellular matrix
FACIT	Fibril-associated collagen with interrupted triple helices
VEGF	Vascular endothelial growth factor
PDGF	Platelet-derived growth factor
Td	Denaturation temperature
EGFR	Epidermal growth factor receptor
MOF	Metal-organic framework
BMSC	Bone marrow mesenchymal stem cells
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
NHS	N-Hydroxysuccinimide (NHS)
DPP-IV	Dipeptidyl peptidase-IV
ASC	Acid-soluble collagen
PSC	Pepsin-soluble collagen
SSC	Salt-soluble collagen
DHT	Dehydrothermal
ACE-I	Angiotensin I-converting enzyme

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Author contributions

QC: Conceptualization, Data curation, Writing—original draft; KT: Conceptualization, Visualization, Project administration, Supervision; YP: Visualization, Writing—original draft; MGA-K: Conceptualization. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Competing interests

Keyong Tang is a member of the editorial board of *Collagen and Leather*. Ying Pei is a member of the young editorial board of *Collagen and Leather*. They

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