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# Advanced application of collagen-based biomaterials in tissue repair and restoration

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## Abstract

In tissue engineering, bioactive materials play an important role, providing structural support, cell regulation and establishing a suitable microenvironment to promote tissue regeneration. As the main component of extracellular matrix, collagen is an important natural bioactive material and it has been widely used in scientific research and clinical applications. Collagen is available from a wide range of animal origin, it can be produced by synthesis or through recombinant protein production systems. The use of pure collagen has inherent disadvantages in terms of physico-chemical properties. For this reason, a processed collagen in different ways can better match the specific requirements as biomaterial for tissue repair. Here, collagen may be used in bone/cartilage regeneration, skin regeneration, cardiovascular repair and other fields, by following different processing methods, including cross-linked collagen, complex, structured collagen, mineralized collagen, carrier and other forms, promoting the development of tissue engineering. This review summarizes a wide range of applications of collagen-based biomaterials and their recent progress in several tissue regeneration fields. Furthermore, the application prospect of bioactive materials based on collagen was outlooked, aiming at inspiring more new progress and advancements in tissue engineering research.

**Keywords:** Collagen, Bioactive material, Tissue engineering, Regeneration, Repair

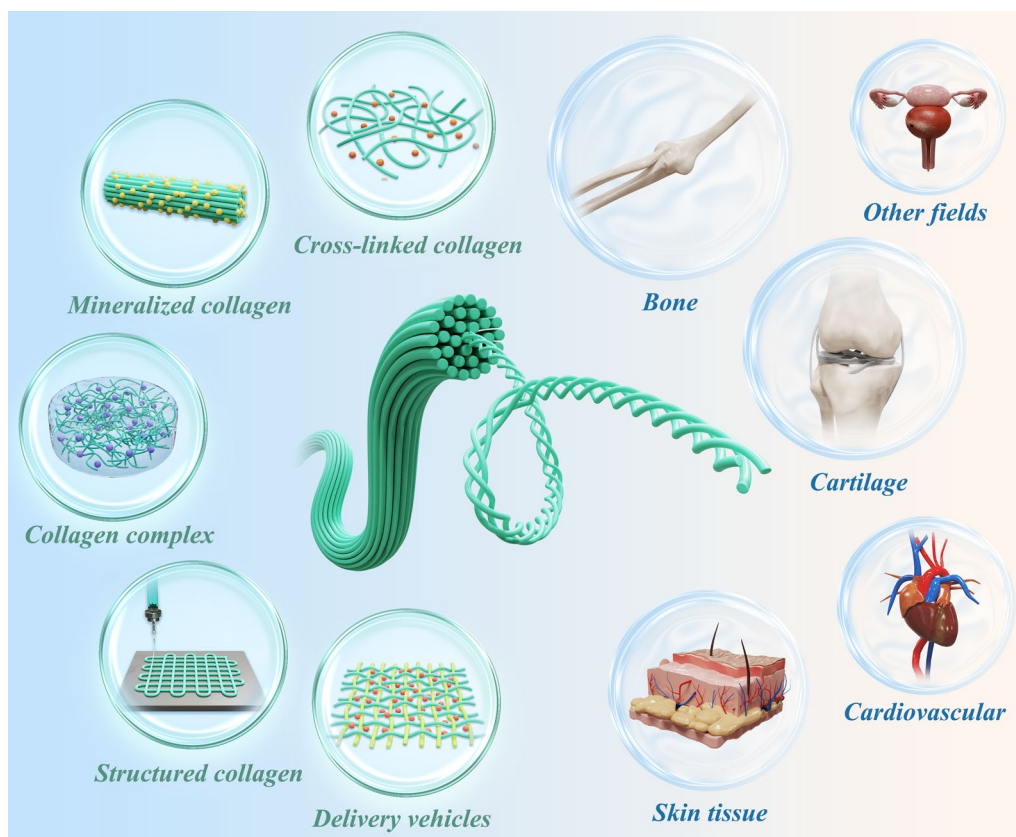
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## Graphical Abstract



## 1 Introduction

In recent years, bioactive materials have played a pivotal role in tissue engineering, which generally provides supporting structures to guide and sustain cells through the use of bioactive ingredients and therapeutic molecules [1]. Bioactive materials should have appropriate mechanical properties, good biocompatibility, and a degradation rate well-matched with the healing process of the body to help restoring the inherent function of tissues [2]. Bioactive materials used to build microstructures that resemble natural tissues [3] can guide morphogenesis, preserve homeostasis and provide tissue healing cues for endogenous/exogenous cells [4], thus creating a micro-environment suitable for cell growth and development. As a natural bioactive material, the extracellular matrix (ECM) plays a significant role in tissue remodeling [5] by providing biological cues that can induce the stimulation of cellular responses, such as cell adhesion, migration and proliferation [6]. Therefore, natural bioactive substrates derived from ECM components can prove to be potential tissue regeneration materials.

As the most abundant bioactive fibrous protein in the ECM, collagen effectively modulates the biological behavior of cells to stimulate native tissue repair [7, 8]. Collagen has a triple helical structure of peptide chains and regulates intracellular signaling and cell activities including morphogenesis, ECM deposition, and tissue remodeling [9, 10] through multiple cell-material interaction pathways [11]. Many cell surface receptors that bind to collagen have been characterized. For instance, cell surface integrins bind to the peptide sequence of collagen and activate the extracellular signal-regulated kinase (ERK) 1/2 mitogen-activated protein kinase (MAPK) signal transduction pathway, promoting cell adhesion, survival, and migration [1]. The amino acid repeats of collagen are specific to immunoglobulin (Ig)-like receptors and G6B-B receptors associated with leukocytes and inhibit immune cell differentiation [12]. Osteoclast associated receptor (OSCAR), an immune receptor specific to the structure of the triple helix peptide, enables the activated T nuclear factor (NFAT) signal transduction, regulates the development of osteoclast (bone resorptive cells), and targets abnormal immune responses [13, 14].

Based on these attributes, collagen has been widely used in the repair of various tissue defects. For instance, mineralized collagen or structured collagen scaffolds have been implanted to induce or guide bone regeneration [15, 16]. Cross-linked collagen, collagen complex, and lyophilized sponge scaffolds have shown promising results in the regeneration of cartilage defects [17, 18]. The use of collagen membrane and drug-loaded collagen scaffolds helps regenerating skin and hair follicles [19]. Collagen-based artificial vascular scaffolds or hydrogels also display great potential in promoting cardiovascular regeneration [20]. In addition, collagen-based bioactive materials also have good application perspectives in gynecology, reproductive medicine [8, 21], plastic repair [22, 23], cornea regeneration [24–26] among others. Collagen scaffolds provide a bionic microenvironment to maintain the structural integrity of regenerated tissues [27], which has an important influence on the cell behavior and tissue repair. As a result, collagen-based biomaterials may be considered as potential candidates for both basic research and clinical applications.

## 2 Basic structure and types of collagen

### 2.1 Sources of collagen

Collagen can be derived from animal skin, Achilles tendon, rat-tail tendon, fish skin, recombinant protein production system, synthetic collagen-like peptides, etc., with good biocompatibility and biodegradation properties. Collagen derived from the skin and Achilles tendon of pigs, cattle, sheep and other animals has been widely used in different studies, owing to the low cost and high yield [28]. However, animal-derived collagen is a highly cross-linked biomaterial with limited solubility [29], large batch to batch variation, immunogenicity, and risk of pathogen transmission [30] which has stimulated the

investigation of marine collagen, human recombinant collagens and collagen-like synthetic peptides. The fish skin contains a large amount of collagen and has a low manufacturing cost [31], meanwhile leads to a better approach for waste utilization and for a cleaner marine environmental [32]. For this reason, marine collagen has attracted great scientific and industrial interest [33] but there are limited studies in tissue engineering [34, 35]. Recombinant collagen are candidates for a variety of medical applications [36] and synthetic collagen-like peptides [37] that mimic the properties of collagen can overcome the batch variability, potential immunogenicity [38] and viruses [39] of animal-derived collagen. However, for recombinant humanized protein, enormous efforts still need to be made to achieve successful translation from bench to clinic [40], considering high cost [41], low yield [42] and susceptibility to enzymatic degradation [43] as compared to animal-derived collagen. The different collagen sources and their characteristics are summarized in Table 1.

### 2.2 Types of collagens

The different types of collagens are named according to the date of discovery [44]. They are mainly classified into different subfamilies based on their structure and function as shown in Table 2. The subfamilies include fibrillar collagens, fibril associated collagens with interrupted triple helices, beaded filament-forming collagen, basement membrane collagens, transmembrane collagens and other collagen with unique functions [4].

The fibrillar collagens constitute about 90% of the total collagen and represent the most abundant and widespread family of collagens including type I, type II, type III, type V and type XI collagen. These collagens are characterized by their ability to assemble into highly oriented

**Table 1** Different sources and properties of collagen

Collagen type	Source	Properties	Disadvantages
Skin collagen	Animal skin (cow, sheep, pig, etc.) Waste leather materials	Low cost High yield	Batch variability Potential immunogenicity Risk of pathogen transmission Low solubility
Achilles tendon collagen	Animal Achilles tendon		
Rat tail collagen	Rat-tail tendon		
Fish skin collagen	Marine fish	Abundant amount in oceans (sustainable) Low cost	Limited studies
Recombinant protein	Recombinant protein production system	Production of special type of collagen Low immune response Overcome batch variability	High cost Low yield Stability issues Proteolytic susceptibility
Synthetic collagen	Synthetic collagen-like peptides	Low immune response Solubility	High cost Low yield Assembly issues Insufficient biological function

**Table 2** Different collagen types organized in various suprastructures. Reproduced with permission from [28]

Suprastructure	Collagen types
Fibril	I, II, III, V, XI, XXIV, XXVII
Fibril associated	IX, XII, XIV, XVI, XIX, XX, XXI, XXII
Network	IV, VI, VIII, X
Anchoring fibrils	VII
Transmembrane collagens	XIII, XVII, XXIII, XXV
Multiplexin	XV, XVIII

supramolecular aggregates with characteristic superstructures. Type I and Type V collagen fibers contribute to the structural skeleton of bone, while type II and XI collagen mainly participate in the fibrous matrix of articular cartilage. Type III collagen is widely distributed in collagen I containing tissues with the exception of bone [45]. Recombinant human collagen type III is also used as three-dimensional (3D) printing ink to produce thin layers crosslinked by EDC:NHS crosslinking for corneal tissue engineering [46]. Type V collagen typically forms heterofibrils with types I and III collagens and contributes to the organic bone matrix, corneal stroma and the interstitial matrix of muscles, liver, lungs, and placenta [47]. Type XI collagen co-distributes with type II collagen in the articular cartilage.

Different types of collagens also affect or cause genetic diseases in humans. For example, a mutation in the exon of type II collagen gene COL2A1 leads to a serine replacement of glycine, resulting in avascular necrosis of the femoral head [48]. Besides, collagen VI is particularly important in skeletal muscle [49]. Collagen VI-related congenital muscular dystrophies is caused by mutations in any of the three genes coding for collagen type VI (COL6A1, COL6A2, COL6A3) [50].

### 2.3 Structure and function of collagen

As shown in Fig. 1a, collagen is made up of three protein chains ( $\alpha$  chains), which are wound together to form a characteristic triple helix. Each alpha ( $\alpha$ ) chain contains about 1000 amino acids with a molecular weight of about 100 kDa, and is composed of a specific set of amino acids repeating sequence  $(\text{Gly-Xaa-Yaa})_n$  [8], where the glycine residues are in every third position. Since glycine is the smallest amino acid with only a hydrogen atom as its side chain, it becomes a part of the center of the super spirochetes without any steric hindrance. This brings the three helical  $\alpha$  chains tightly together to form the final super spirochetes with hydrophobic cores. The other two amino acids in Xaa and Yaa positions are often proline and hydroxyproline.

Collagen is cleaved from precursor molecule (procollagen) as shown in Fig. 1b [51–53]. A key regulatory step in collagen assembly is the C-terminal proteolytic processing of soluble procollagen precursors [54]. Procollagen N-proteinase and procollagen C-proteinase are essential for procollagen processing. The C-proteinase specifically cleaves native and denatured types I, II and III procollagens [55]. The N-proteinase has the unusual property of cleaving the N-propeptides from type I and type II procollagens if the proteins are in a native conformation, but not if the proteins are partially unfolded so that the N-telopeptides are no longer in a hair-pin configuration.

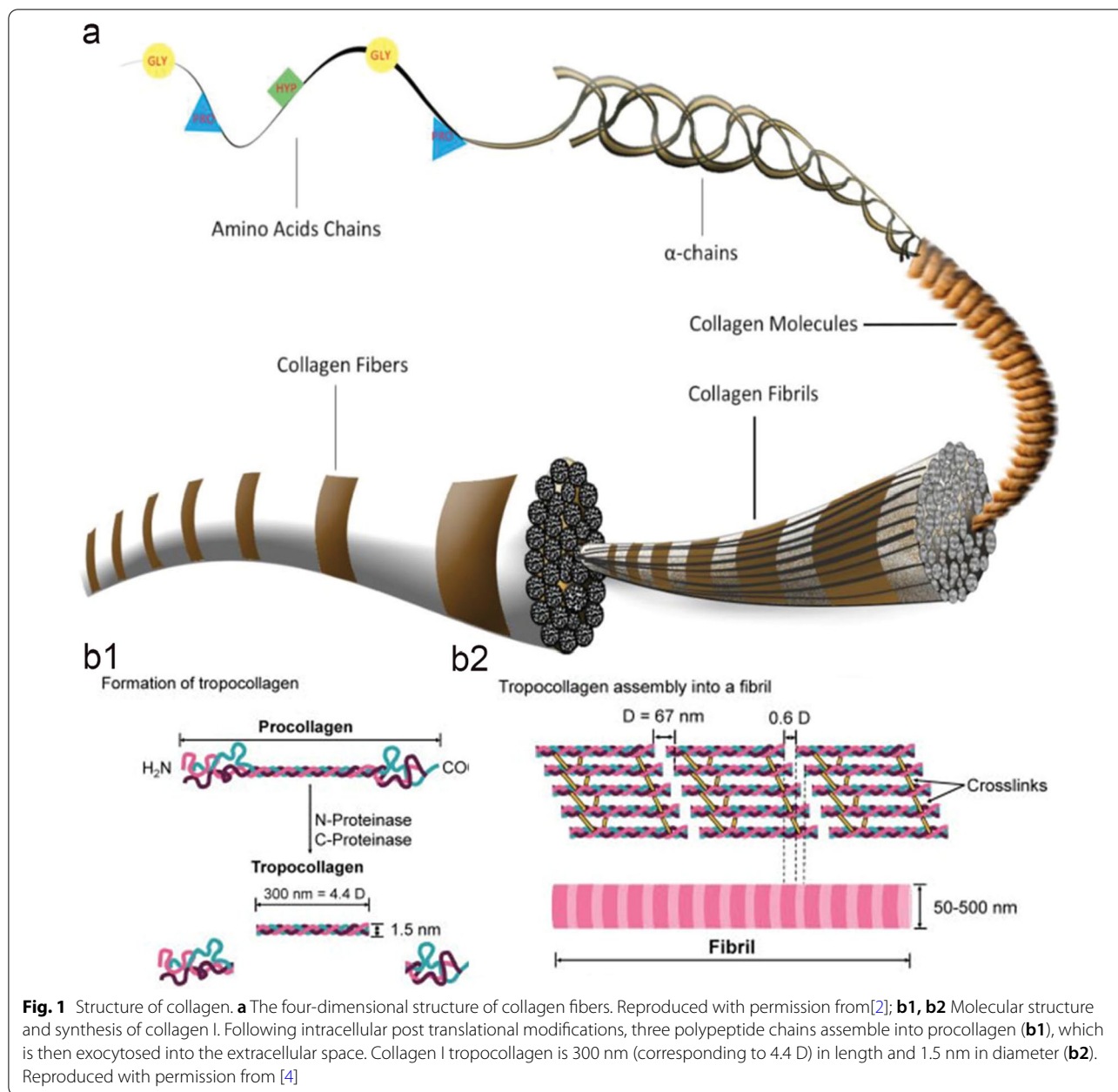
Collagen maintains the structural integrity of tissues and organs and acts as the main functional skeleton in connective tissues, especially bone and cartilage [56]. In addition, collagen interacts with cells mediated by its specific peptide repeat unit and triple helix structure, and regulates their adhesion, proliferation, differentiation, and signal response [44] by binding to specific receptors such as integrins, glycoproteins, and proteoglycan receptors [57, 58]. Collagen further contributes to the local storage and delivery of loaded and endogenous growth factors and cytokines, providing a suitable microenvironment for exogenous/endogenous cells involved in organ development, wound healing, and tissue repair [59].

### 3 Development of collagen-based biomaterials

Natural collagen has characteristics for befittingly matching with tissue. However, due to the destruction of the natural assembly structure and cross-linking of collagen in the process of extraction, purification and synthesis [60], the mechanical properties and stability of extracted collagen are lower than its natural counterpart, resulting in its low durability and limited potentiality in biotechnology applications [61]. Therefore, a variety of different strategies have been pursued to reconstruct and enhance the structure and properties of collagen.

#### 3.1 Cross-linked collagen

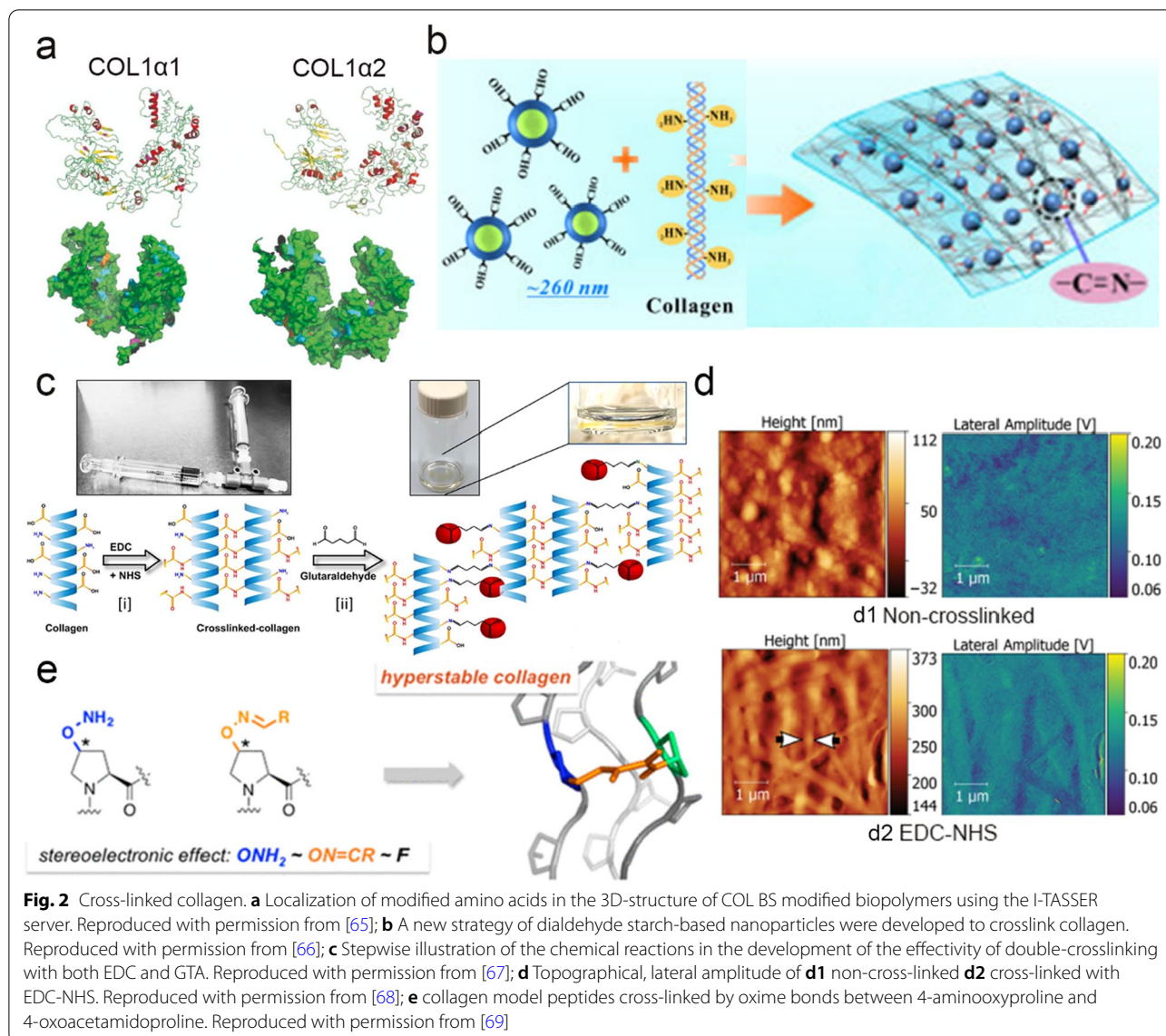
Considering that the natural cross-linking pathway of collagen does not occur in vitro [62], exogenous cross-linking is introduced to optimize collagen-based materials. The introduction of additional cross-links prevents collagen molecules from sliding against each other under stress [63], thereby increasing the mechanical strength of collagen fibers. Further, the potential modification of collagen is limited by its structural complexity and biocompatibility requirements [64]. Common cross-linking methods are presented in Fig. 2. However, there is currently no standardized cross-linking methods to prepare a strong, biocompatible collagen matrix.



### 3.1.1 Physical cross-linking of collagen

Physical cross-linking using ionizing radiation, ultraviolet (UV) light treatment, dehydrogenation heat treatment (DHT), or photo-oxidation is generally considered to be a simple and safe method to prepare a cross-linked collagen matrix [61]. For example, Pien et al. described the introduction of modified photo-crosslinkable groups into the 3D structure of proteins (Fig. 2a). Proteomic analysis was used to quantify photo-crosslinking through the identification and localization of modified peptides, which is critical for repeatability and regulation

of biomaterials [65]. Wang et al. used UV radiation and DHT to improve the properties of collagen casings (a form of collagen film). UV treatment, DHT, and their combination significantly increased the tensile strength and decreased the elongation at break of collagen casings, among which DHT had a significant effect [70]. In summary, physical cross-linking successfully enables the development of biomaterials with better mechanical properties and excellent biocompatibility without the use of exogenous toxic chemicals [45]. However, physical cross-linking are often associated with collagen



denaturation (especially the DHT treatment), imposing the need for introduction of chemical crosslinkers [30].

### 3.1.2 Chemical cross-linking of collagen

The most widely used chemical crosslinking agents are aldehydes. Glutaraldehyde (GTA) has been shown to extensively stabilize collagen materials because of its self-polymerization capacity but still controversial due to its toxicity [71]. For this reason, research interests are focusing on alternative strategies. Xu et al. [66] developed dialdehyde cross-linked collagen with starch-based nanoparticles as a new strategy. Dialdehyde cholesterol modified starch self-assembled into nanoparticles and cross-linked with collagen to fabricate collagen hydrogels as shown in Fig. 2b. Islam et al. [67] evaluated the effect

of double-crosslinking with both EDC and GTA together with the capability of sodium metabisulfite (SM) and sodium borohydride (SB) to neutralize the toxicity and restore biocompatibility after cross-linking, as reported in Fig. 2c. Nair et al. [68] employed 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide (EDC-NHS) to form structurally and mechanically stable collagen scaffolds and investigated its effect on collagen piezoelectricity. The topography and lateral amplitude was shown in Fig. 2d. Hentzen et al. reported the cross-linking of model collagen peptides by oxime bonds between 4-aminooxyproline and 4-oxoacetamidoproline placed in coplanar Xaa and Yaa positions of neighboring strands (Fig. 2e) [69]. Compared to GTA, these natural cross-linking agents had lower toxicity and better

biocompatibility but possessed limitations including storage instability and low degree of cross-linking [71]. For example, naturally-derived chemical crosslinking agents, such as genipin, are also attracting research attention, owing to their interesting pharmacological properties (i.e., anti-inflammatory, anti-angiogenic, anti-cancer, among others) [72].

Click chemistry approach provides new ideas for collagen architectures and assembly behaviors. These conjugate strategies with collagen mimetic peptides as the building blocks present exciting stimuli responsive or spontaneously assembly behavior [73]. Byrne et al. [74] established modular synthesis of end-stapled homo- and hetero-triple helical peptides, generating “clicked” macro-assemblies with enhanced thermal stability. Collagen can also be functionalized by click chemistry to play a greater role in tissue regeneration. Lee et al. [75] studied the chemical coupling of growth factors to collagen using click chemistry and discovered that it had significant effects on epithelial cell attachment and proliferation. Click chemistry-based reaction can connect, more accurately, the various functional groups, so that the cross-linking reaction can proceed in the direction as expected.

### 3.1.3 Enzyme-induced cross-linking of collagen

Enzyme cross-linking has emerged in recent years because of its excellent specificity and precise reaction kinetics [76]. Perez-Puyana et al. [77] developed a hybrid scaffold made from the mixture of two biopolymers (collagen and chitosan) and compared the effect of GTA as a cross-linking agent with three different cross-linking methods (chemical: genipin; physical: temperature and enzymatic: transglutaminase) to look for a promising candidate to substitute it. Enzymatic cross-linking avoids the disadvantages of physical and chemical cross-linking, but it is expensive.

## 3.2 Mineralized collagen

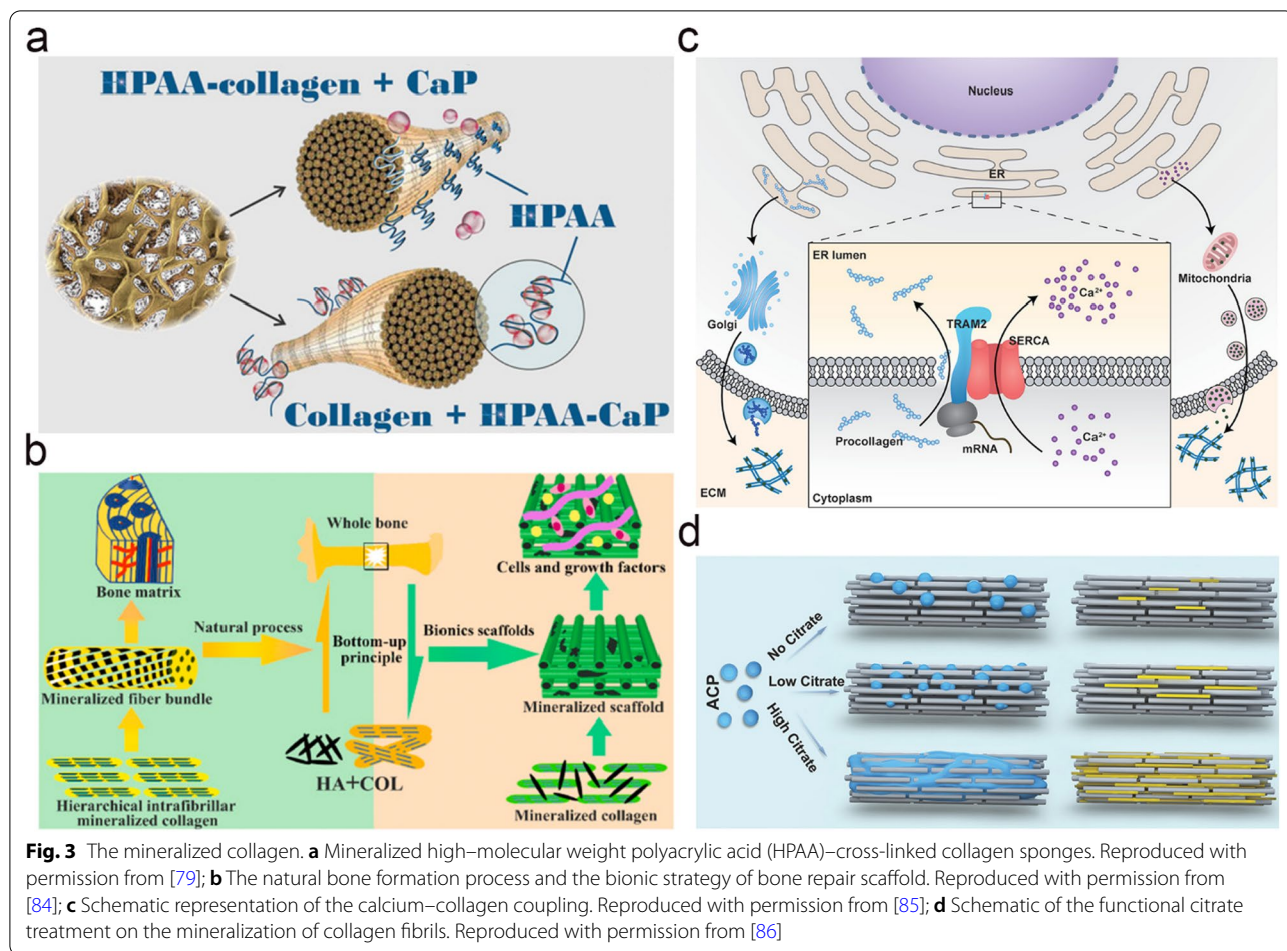
Mineralized collagen is an organic–inorganic composite material formed from collagen molecules and nano-calcium phosphate minerals [78]. It is used as a universal template in biomineralization [79]. The inorganic components closely related to collagen are limited to a few minerals: amorphous silica, calcium carbonate polycrystalline, and carbonate apatite [80] and may precipitate within (intrafibrillar mineralization) and between collagen fibrils (interfibrillar/extrafibrillar mineralization) [81]. As bone largely consists of intrafibrillar hydroxyapatite (HAp) crystals, the formation of intrafibrillar HAp in a collagen matrix was investigated [82]. During intrafibrillar mineralization, large contractile forces occur within the collagen regardless of the mineral type, thus

giving bone its unusual combination of mechanical properties [83].

Biomineralized collagen composites have the potential to be used as a substitute for current synthetic bone implants by providing biomimetic components that are very similar to natural bone [87]. More effective mineralization methods and detailed mineralization mechanisms are under investigation. Song et al. found that a polyelectrolyte polyacrylic acid (PAA) is capable of caching calcium and hydrogen phosphate ion complexes into chain-like aggregates along the surface of the macromolecule (Fig. 3a) when it is introduced into a supersaturated calcium phosphate (CaP) mineralization medium, as well as concentrating the precursors for more efficacious intrafibrillar mineralization [79]. Zhang et al. [85] analyzed the biological mineralization of natural bone and found a correlation between calcium concentration and collagen production (Fig. 3c). The translocation-associated membrane protein 2 (TRAM2) was identified as an intermediate factor that modulates the activity of calcium-ATPase type 2 in the sarco-/endoplasmic reticulum (SERCA2b) to couple calcium enrichment with collagen biosynthesis, to be involved in bone matrix mineralization. Shao et al. found that citrate molecules adsorbed on collagen fibrils could significantly reduce the interface energy between biological matrix and amorphous calcium phosphate (ACP) precursor (Fig. 3d), enhance their wetting effect during early-stage biomineralization, and successfully promote the formation of HAp fibrils to generate inorganic–organic complexes. This finding demonstrates the importance of interfacial controls in biomineralization [86]. As presented in Fig. 3b, the further study in the design of mineralized collagen should have a satisfactory analog structure and appropriate biological properties for bone regeneration [84].

## 3.3 Collagen-based blends

Blends can be prepared based on synthetic and natural polymers as well as two (or more) biopolymers. It is expected that blending two polymers can lead to the development of a new class of materials with improved mechanical properties and biocompatibility when compared with a single component material [29]. Collagen has certain limitations related to its applications in tissue repair, such as strong contraction, weak mechanical properties, and poor cell phenotypic orientation [4]. Therefore, collagen-based blends are expected to prepare complex hydrogels with improved practical performances. To this aim, collagen-based blends have been widely used to study the interaction of cells with their microenvironment and as scaffolds for biomedical and tissue engineering applications [92, 93].



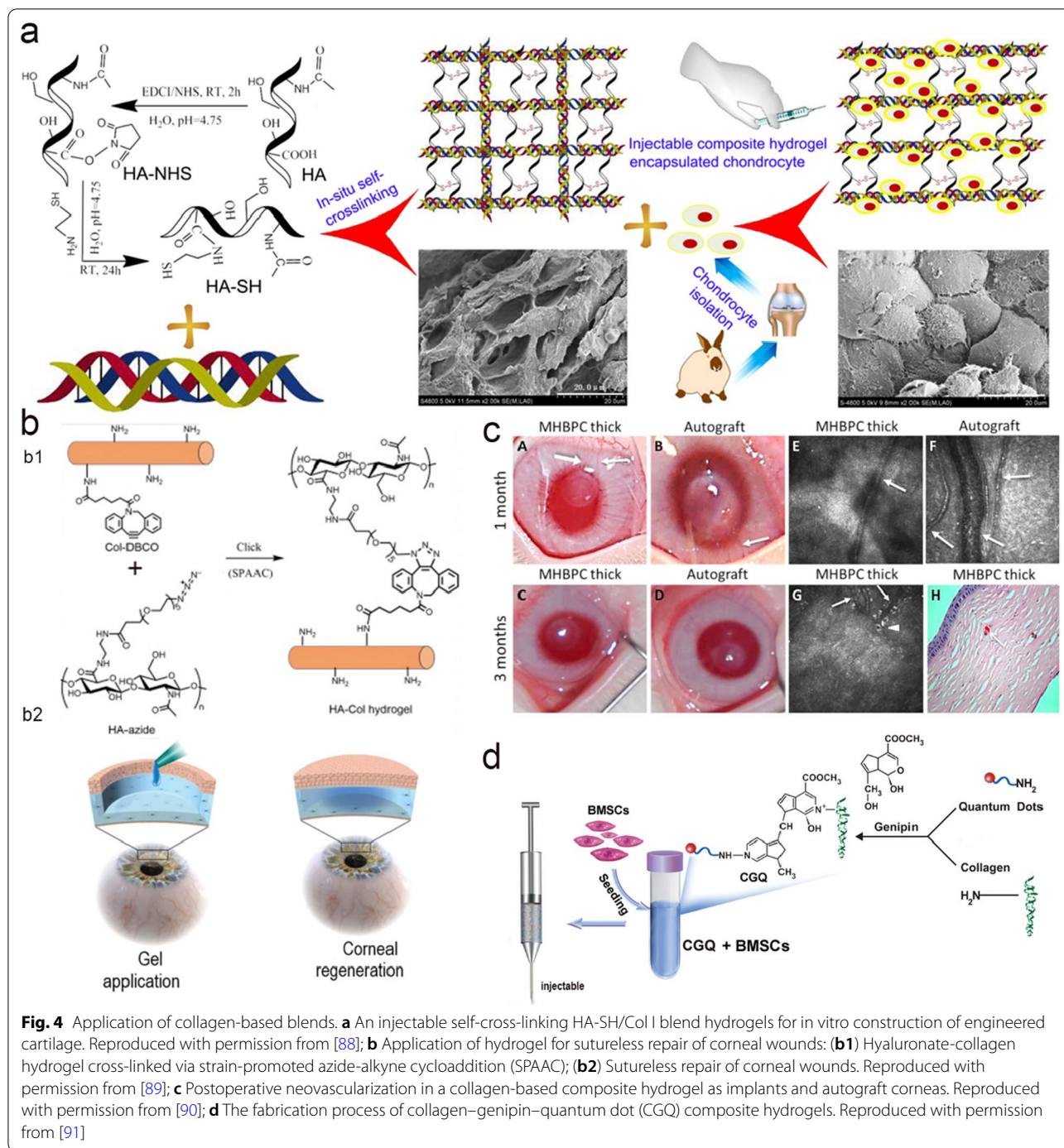
Examples of different collagen-based blends are shown in Fig. 4. For instance, Chen et al. developed an injectable self-crosslinking hyaluronic acid (HA-SH)/type I collagen (Col I) blend hydrogels for in vitro development of an engineered cartilage as present in Fig. 4a [88]. For cornea regeneration, Chen et al. designed a bio-orthogonal hyaluronic acid-collagen hydrogel for sutureless corneal defect repair (Fig. 4b). The results showed improved mechanical properties and excellent cytocompatibility and support for epithelialization in vitro and in vivo [89]. Rafat et al. [90] developed a collagen-based composite hydrogels (Fig. 4c) as implants to restore corneal transparency while serving as a possible reservoir for cells and drugs. Zheng et al. designed a genipin-cross-linked injectable composite collagen hydrogel as shown in Fig. 4d, which was used as a scaffold for incorporating bone marrow-derived mesenchymal stem cells (BMSC) and cadmium selenide quantum dots for cartilage repair [91]. It has been emphasized that collagen plays a significant role in tissue engineering, and thus, numerous products based on this material have been developed so far.

### 3.4 Structured collagen

Over the past two decades, problems associated with regenerating natural collagen fibers include the inability to achieve sufficient tensile strength and replicate or reproduce the internal fibrillary structure due to the loss of properties of the hierarchies consistent with natural collagen [96]. Modern technology enables to make highly ordered collagen scaffolds or a matrix with a structure similar to the matrix present in living organisms, as shown in Fig. 5 [98].

The ability of the electrospinning to fabricate custom-built nanofibers (Fig. 5b) from collagen-based composites may be the main reason that made it the most applied method to mimic the structure of the collagen in native tissues [96]. However, there are several challenges for collagen electrospinning, such as poor mechanical strength, reduced elasticity, high hydrophilicity of the obtained fibers, and difficulty in solubilizing collagen while preserving its native tertiary structure [95]. Indeed, natural collagen fibers may lose their structural characteristics while processing. For this reason, further modifications

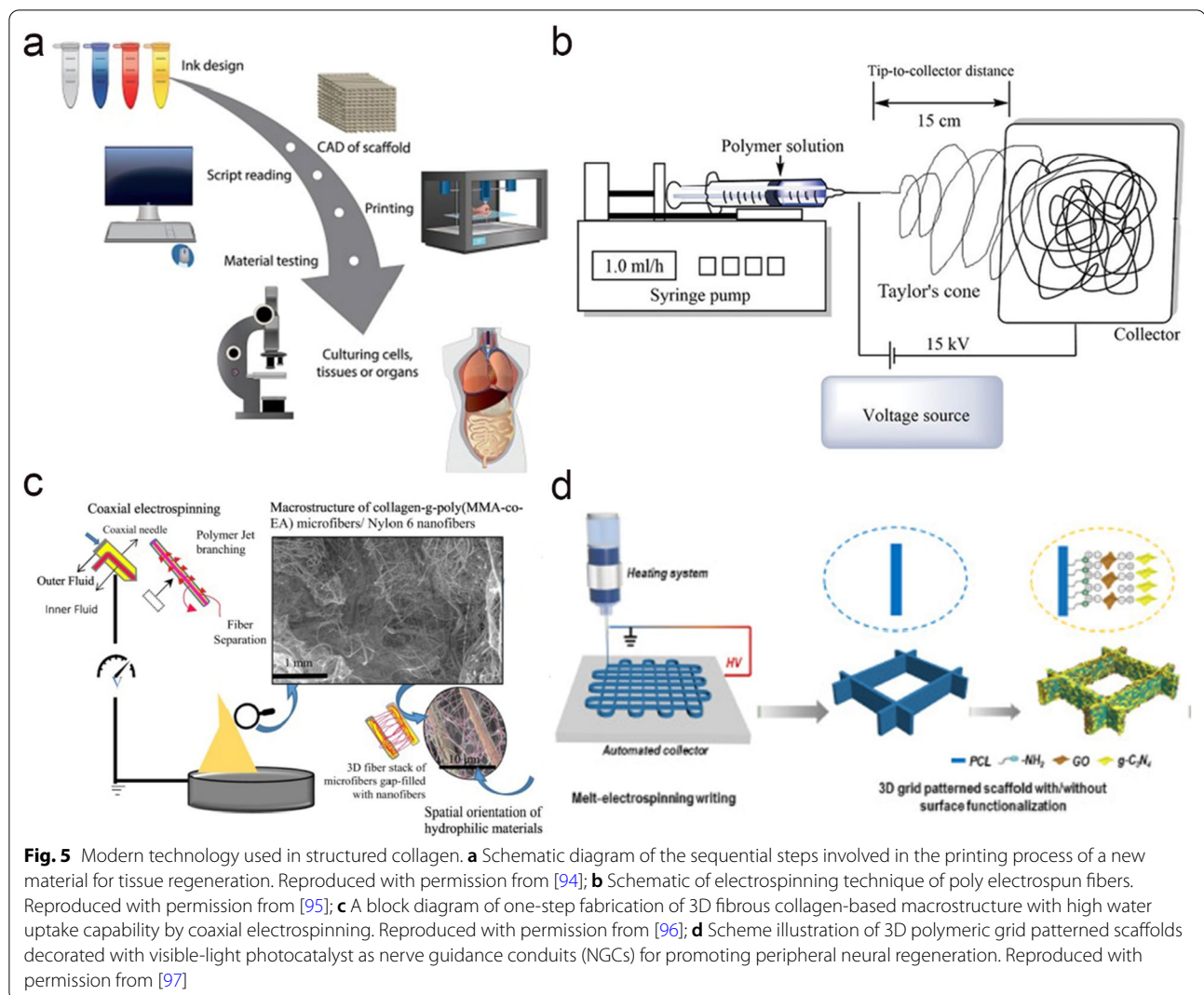




are warranted to counter the negative effects of processing [96].

3D printing is one of the most famous additive manufacturing technologies in the field of tissue and organ bio-manufacturing, and the sequential steps involved in the printing process are shown in Fig. 5a [94]. Compared with traditional techniques, 3D printing offers

the possibility of creating replicable, customizable and functional structures with precisely controlled composition and microstructure that can effectively promote the regeneration of different tissues [99]. However, due to the low mechanical stability, collagen is typically printed in preformed molds, inside other materials



serving as a support or stabilized by additional cross-linking steps after printing [100, 101].

The melt near-field direct writing technology overcomes some of the defects of spinning methods in terms of disorder and control and enables the fabrication of micro/-nanowires with precisely controlled morphology (Fig. 5d) [97]. Further optimizations in the design of structured collagen are required to reproduce the natural organization and biomimetic function and achieve better results in tissue regeneration applications.

### 3.5 Collagen-based delivery vehicles

Collagen suspensions can be easily prepared and used as delivery vectors for macro- and micro drug molecules. Methods such as supercritical fluid extraction, electrospraying, spray drying, layer-by-layer self-assembly, lyophilization and microemulsification can be used to introduce drugs into delivery systems [102]. Sun et al.

[103] developed a recombinant SDF-1 $\alpha$  containing collagen-binding domain which could specifically bind to collagen and achieve controlled release of SDF-1 $\alpha$  for in situ tendon regeneration. Kanematsu et al. [104] investigated the use of natural and synthetic collagenous matrices as carriers of exogenous growth factors, which could actively promote tissue regeneration. Collagen-based delivery systems also need to possess optimal degradation rate and drug dispersion properties to achieve better effects [105].

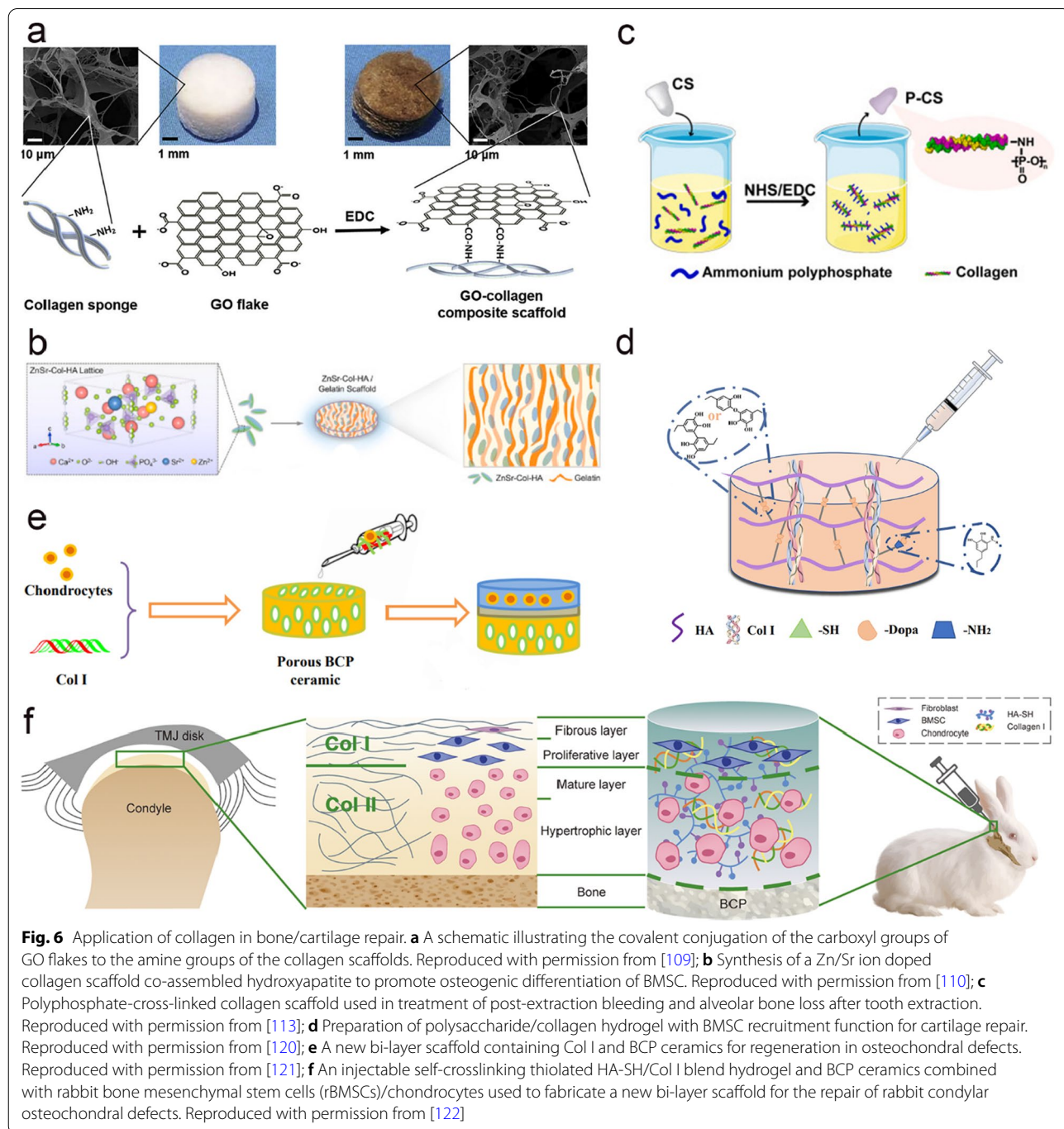
## 4 Applications of collagen-based biomaterials

### 4.1 Bone regeneration

The excellent mechanical properties of bone come from the orderly embedding of nano mineral crystals into the collagen matrix, thus forming a complex ordered hierarchical structure [106]. The organic matrix of bone mainly comprises collagen fibrils, specifically type I collagen. Therefore, type I collagen is widely used in bone repair

as a naturally occurring matrix in the form of hydrogel and scaffold [107]. However, the low mechanical strength and limited osteogenicity of collagen limit its wide application in bone regeneration [15]. Therefore, by adding some bioceramics similar to the inorganic components of natural bone [108], the mechanical properties, porosity, structural stability, osteogenic/osteoinductive features, and other properties of the collagen matrix can

be greatly improved [16]. For instance, Kang et al. fabricated graphene oxide (GO)-collagen (GO-COL) scaffold crosslinked by EDC as shown in Fig. 6a for osteogenic differentiation of human mesenchymal stem cells [109]. Zhong et al. [110] developed a Zn/Sr ion doped collagen scaffold co-assembled HAp as reported in Fig. 6b to induce favorable bone immune microenvironment by stimulating macrophages to promote osteogenic



**Fig. 6** Application of collagen in bone/cartilage repair. **a** A schematic illustrating the covalent conjugation of the carboxyl groups of GO flakes to the amine groups of the collagen scaffolds. Reproduced with permission from [109]; **b** Synthesis of a Zn/Sr ion doped collagen scaffold co-assembled hydroxyapatite to promote osteogenic differentiation of BMSC. Reproduced with permission from [110]; **c** Polyphosphate-cross-linked collagen scaffold used in treatment of post-extraction bleeding and alveolar bone loss after tooth extraction. Reproduced with permission from [113]; **d** Preparation of polysaccharide/collagen hydrogel with BMSC recruitment function for cartilage repair. Reproduced with permission from [120]; **e** A new bi-layer scaffold containing Col I and BCP ceramics for regeneration in osteochondral defects. Reproduced with permission from [121]; **f** An injectable self-crosslinking thiolated HA-SH/Col I blend hydrogel and BCP ceramics combined with rabbit bone mesenchymal stem cells (rBMSCs)/chondrocytes used to fabricate a new bi-layer scaffold for the repair of rabbit condylar osteochondral defects. Reproduced with permission from [122]

differentiation of BMSCs. This biomimetic combination of organic and inorganic components mimics the biochemical and biophysical properties of the bone matrix [111] and can be used to develop promising candidates for applications in bone regeneration.

Dentine, a bonelike matrix, is also a hard tissue of mineralized collagen [112]. Therefore, collagen-based materials are also widely used in dentistry. Gu et al. developed a collagen scaffold covalently cross-linked using polyphosphate as shown in Fig. 6c with better hemostasis and bone regeneration ability [113]. It controlled the bleeding and enabled alveolar bone retention after tooth extraction. Furthermore, absorbable barrier collagen membranes have been widely used for regeneration of periodontal defects in oral surgery because of their biocompatibility and ability to promote wound healing and help to avoid a second surgery [114].

#### 4.2 Cartilage regeneration

Hyaline articular cartilage lacks blood vessels, lymphatics, and nerves, and has cells with a low replication potential, which limits its ability to heal [115]. As the main component of articular cartilage, collagen acts as structural and functional support [116] and is widely used in cartilage repair. Collagen-based biomaterials are generally used in the form of hydrogel or freeze-dried scaffolds, supplemented by seed cells or growth factors to form a suitable microenvironment for cartilage tissue regeneration [117]. Type II collagen is the main constituent of hyaline cartilage [118]. However, extracted type II collagen is usually not gelled by itself and is added as a cue to induce tissue regeneration [119].

In terms of cartilage repair, collagen-based scaffolds play an important role in cartilage tissue engineering. Li et al. prepared injectable cartilage repair hydrogels by polysaccharides and collagen, which had good effects on recruiting stem cells and promoting the chondrogenic differentiation of stem cells (Fig. 6d) [120]. Collagen was also used for subchondral bone repair and osteochondral full-layer repair. Cai et al. used Col I and biphasic calcium phosphate (BCP) ceramics to fabricate a new bi-layer scaffold for regeneration in osteochondral defects (Fig. 6e) [121]. Wang et al. developed an injectable self-crosslinking thiolated HA-SH/Col I blend hydrogel and BCP ceramics, combined with rabbit bone mesenchymal stem cells (rBMSCs)/chondrocytes used to fabricate a new bi-layer scaffold to simulate specific structure of rabbit condylar osteochondral defects as reported in Fig. 6f [122]. Levingstone et al. developed a multi-layer bionic collagen scaffold and implanted the scaffold into goat joints to study the long-term ability to repair osteochondral defects in large animal models [123]. Studies have shown that composite scaffolds generally have

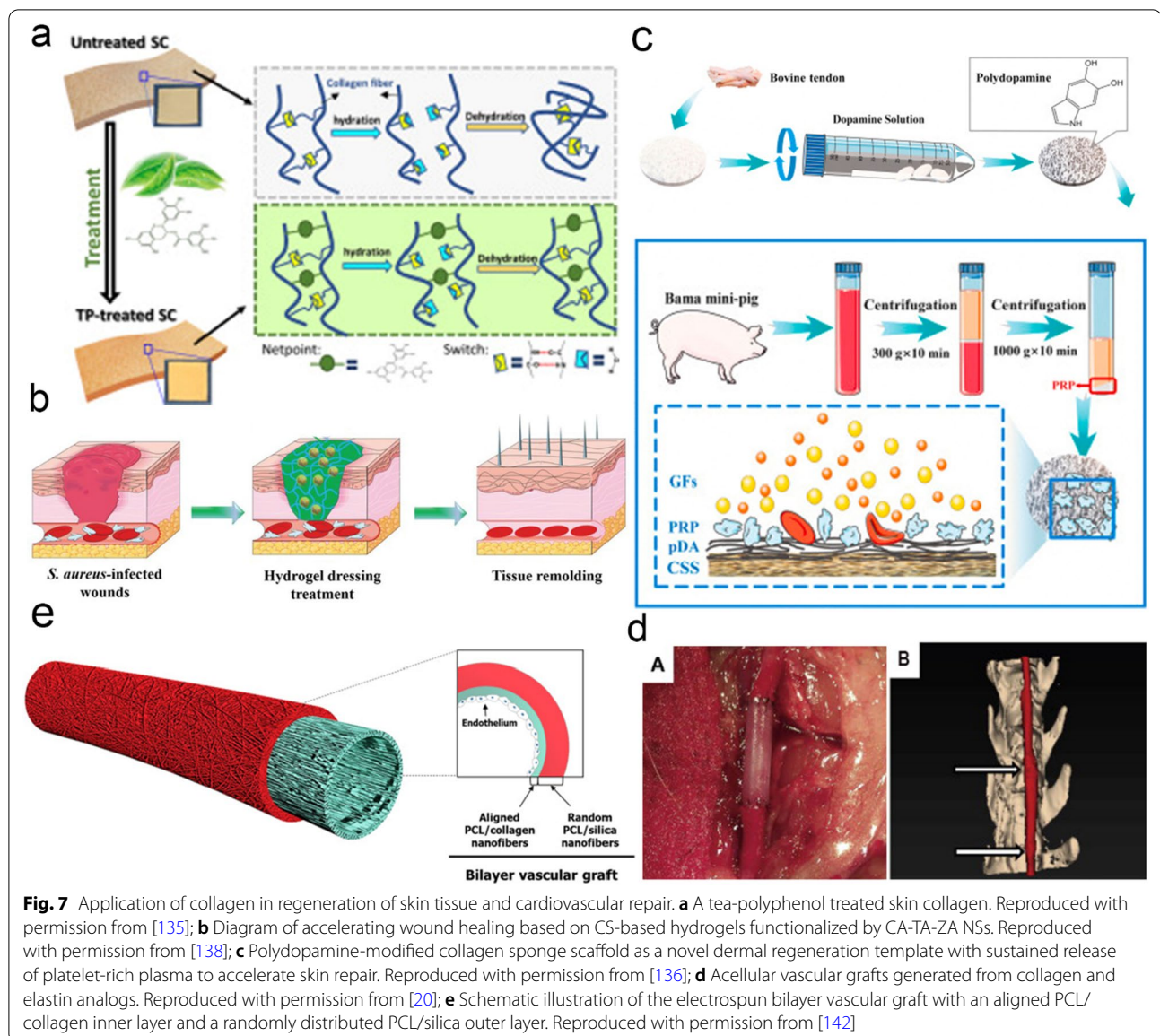
a better potential to induce cartilage regeneration than single-component scaffolds [124]. The composition and structural design of the scaffolds based on the physiological structure of cartilage aims at long-term and effective repair.

#### 4.3 Skin tissue regeneration

Intricate bundles of collagen create a 3D network of fibers in the human skin [19]. Collagen regulates dermal matrix assembly and fibroblast movement, in turn fibroblast and derived matrix metalloproteinase (MMP)-14 regulate collagen homeostasis in the skin [125, 126]. Procollagen types I, III and VI are the major collagenous products of cultured human skin fibroblasts [127]. Collagen XII is a homotrimer with short collagenous domains that confer binding to the surface of collagen I-containing fibrils and extended flexible arms, therefore helps to maintain collagen suprastructure and to absorb stress [128].

Full-thickness skin defects are difficult to heal naturally due to their size and loss of vasculature and are highly susceptible to bacterial infection [129]. Usually, hemostatic sponges and wound dressings are used to stop bleeding and form a barrier with the outside. Traditional dressings, such as sterile gauze, medical skimmed cotton etc. can only serve as a simple physical barrier, and cannot effectively promote skin wound healing, and traditional wound dressings are easy to adhere to the wound, causing secondary damage when changing the dressing [130]. Collagen wound dressings are currently used for burns [131], trauma, infectious and surgical skin injuries, and chronic trauma procedures due to their anti-inflammatory, analgesic, and hemostatic effects, biodegradability, absorbability, strong water absorption capacity, which can help providing support for cell attachment [132–134]. To cite an example, Han et al. developed a tea-polyphenol treated skin collagen achieving hydro-structural adaptability, mechanical intelligence and shape-memory advantages for further skin-care products as shown in Fig. 7a [135]. Zheng et al. [136] used the polydopamine-modified collagen sponge scaffold as a new dermal regeneration template to continuously release platelet-rich plasma to accelerate skin repair (Fig. 7c). Yan et al. [137] prepared a *Bletilla striata* polysaccharide modified collagen composite sponge and studied its hemostatic properties.

Many skin tissue engineering methods employ easy-to-handle and sizing methods to enhance the mechanical strength of collagen. By resisting shrinkage, the enhanced collagen hydrogel can maintain coverage of the wound throughout the healing process as shown in Fig. 7b [138, 139]. Further, the compression of collagen hydrogels following cell encapsulation can also improve their mechanical strength, stiffness, and tensile strength values closer



**Fig. 7** Application of collagen in regeneration of skin tissue and cardiovascular repair. **a** A tea-polyphenol treated skin collagen. Reproduced with permission from [135]; **b** Diagram of accelerating wound healing based on CS-based hydrogels functionalized by CA-TA-ZA NSs. Reproduced with permission from [138]; **c** Polydopamine-modified collagen sponge scaffold as a novel dermal regeneration template with sustained release of platelet-rich plasma to accelerate skin repair. Reproduced with permission from [136]; **d** Cellular vascular grafts generated from collagen and elastin analogs. Reproduced with permission from [20]; **e** Schematic illustration of the electrospun bilayer vascular graft with an aligned PCL/collagen inner layer and a randomly distributed PCL/silica outer layer. Reproduced with permission from [142]

to those of natural skin. These hydrogels could also maintain high cellular viability, which is more favorable to the response of dermal fibroblasts and keratinocytes [140, 141].

#### 4.4 Cardiovascular repair

Collagen's hypoallergenic nature enables its application as a vascular implant (Fig. 7d) or stent, a component of artificial heart valves and a coating for cardiovascular stents [143]. However, the thrombogenic properties and in vivo calcification of collagen limit its application [20], and collagen-based stents generally do not meet the mechanical requirements of a dynamic cardiovascular environment. To address some of the

mechanical limitations of natural polymers like collagen, composites have been developed with improved strength of while maintaining biological activity. Heydarkhan-Hagvall et al. designed a 3D electrospinning hybrid scaffold based on collagen/elastin/polycaprolactone (PCL) for cardiovascular tissue engineering [144]. Park et al. [142] developed a small-diameter PCL vascular graft (Fig. 7e) with a functional, bilayered PCL/collagen and PCL/silica nanofibrous structure and a composition that enables a suitable healing process and gradual degradation/replacement by natural blood vessels. The main problem with creating artificial arteries outside the body is that arteries have a complex natural structure that requires materials with

adequate strength and elasticity, as well as durability for long-term use after implantation.

#### 4.5 Other areas of tissue repair

Different adaptive forms of collagen applied in different tissue repair fields, as already mentioned, are summarized in Table 3. Besides, collagen-based bioactive materials also have good application perspectives in the fields of gynecology, reproductive medicine, plastic repair and cornea regeneration. Collagen has an impact on the important biological functions and stability of ECM, which in turn has a dynamic influence on the menstrual cycle and pregnancy. This makes collagen advantageous in gynecology and genitourinary field. In the 1990s, Jackson et al. [145] found that genitourinary prolapse was associated with a reduction in total collagen content and a decrease in collagen solubility, and suggested the development of agents to inhibit collagenolytic activity to help the treatment of this condition. In recent years, collagen-based materials have been widely developed. Collagen-based biomaterials, such as collagen hydrogels, decellularized ECM (dECM) and bioengineering techniques, 3D bioprinting of collagen, may replace hormone therapy and hold promise in reproductive organ reconstruction. Grimm et al. [146] performed clinical trials to evaluate the efficacy of a collagen-fibrin patch for the prevention of symptomatic lymphoceles after pelvic lymphadenectomy in women with gynecologic malignancies. Furthermore, as a natural component of skin, collagen are regarded as gold standard in dermal tissue reconstruction [147] and have been used in plastic surgery and cosmetic surgery for many years, and it is still in continuous innovation and development.

#### 5 Future perspectives

Collagen has been widely used in bone/cartilage regeneration, skin repair, cardiovascular repair and other regenerative medicine applications. However, different sources and production methods lead to large variation in collagen batches, which has a serious impact on the functional stability of collagen products. In addition, the price of collagen is still very high, and the potential immunogenicity to the implanted objects warrants an urgent need to develop an effective standard production methodology and testing. The disadvantage of large batch variation of animal-derived collagen can be circumvented using biocompatible recombinant human collagen with low variability and immunogenicity. Recombinant collagen is particularly attractive as it enables the production and purification of uncommon collagen types. However, recombinant human collagen also has a few disadvantages, such as high cost, low yield and stability issues. In recent years, marine collagen sourced from marine organisms, such as fish skin and scales, has come into the spotlight. The source of marine collagen is sustainable and environmentally friendly, but its specific properties and applications need to be explored further.

Another hassle is the damage of the natural structure of collagen during the extraction process, which results in poor mechanical properties and weak gel-forming capability, thereby limiting its use in tissue engineering applications. Cross-linked collagen, collagen-based blends, mineralized collagen and structured collagen are optimized in different ways to improve the performance of collagen and meet the functional requirements in clinical applications. Cross-linking collagen can effectively improve its mechanical properties and stability, but the cross-linking method should be carefully chosen to avoid toxicity and inadvertent changes

**Table 3** Adaptive morphology and characteristics of collagen in different application fields

Application field	Collagen form	Characteristics
Bone	Mineralized collagen Additive manufacturing scaffolds	Suitable inorganic/organic phase Shape designable, Mechanical strength
Cartilage	Hydrogel Freeze-dried sponge Cross-linked collagen	Bionic cartilage Cartilage differentiation Long-term repair
Skin Tissue	Hydrogel Skin protectant textiles Collagen wound dressings Concentrated collagen	Anti-inflammatory Resistance to shrinkage Improved mechanical properties Drug treatment
Cardiovascular	Hydrogel Collagen membrane/ film Coating	Mechanical requirements (strength and elasticity) Durability Calcification resistance

to the structure of collagen itself. Currently, there are no standard cross-linking agents available. Compared to single-component collagen, composite collagen scaffolds provide a more suitable microenvironment for tissue regeneration. However, the design of composite materials needs to be further optimized. Mineralized collagen contains both organic and inorganic components that mimic natural bone structure, but the mineralization process in vivo needs a precise regulation. Several novel technologies have been reported to develop scaffold materials, using structured collagen suitable for personalized medicine. Further research is required to achieve careful control at the microstructure level of structured collagen to preserve the ligand sites along the collagen chain. With the development of preparation processes that preserve the inherent collagen structure and chemistry, these collagen-based materials will emerge as versatile candidates for breakthrough applications in the field of tissue regeneration, complex structure mimic construction, and drug delivery.

#### Abbreviations

ECM: Extracellular matrix; ERK: Extracellular signal-regulated kinase; 3D: Three-dimensional; MAPK: Mitogen-activated protein kinase; Ig: Immunoglobulin; OSCAR: Osteoclast associated receptor; NFAT: Activated T nuclear factor; UV: Ultraviolet; DHT: Dehydrogenation heat treatment; GTA: Glutaraldehyde; EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS: N-hydroxysuccinimide; SM: Sodium metabisulfite; SB: Sodium borohydride; HAp: Hydroxyapatite; PAA: Polyacrylic acid; CaP: Calcium phosphate; HPAA: High-molecular weight polyacrylic acid; TRAM2: Translocation-associated membrane protein 2; SERCA2b: Calcium-ATPase type 2 in the sarco-/endoplasmic reticulum; ACP: Amorphous calcium phosphate; SPAAC: Strain-promoted azide-alkyne cycloaddition; BMSC: Bone marrow-derived mesenchymal stem cells; HA: Hyaluronic acid; Col I: Type I collagen; BCP: Biphasic calcium phosphate; NGCs: Nerve guidance conduits; GO: Graphene oxide; HA-SH: Self-crosslinking hyaluronic acid; rBM-SCs: Rabbit bone mesenchymal stem cells; PCL: Poly-ε-caprolactone; dECM: Decellularized ECM.

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#### Author contributions

YS and UD made substantial contributions to the conception, design and revision of this review. JZ wrote the manuscript. ZL and YZ downloaded and summarized related literatures. GL, AR and JL designed and retouched all figures and tables. YF and XZ participated in the corrections and the design of figures. All authors read and approved the final manuscript.

#### Availability of data and materials

The datasets analyzed in this review are available as quoted and listed in the "References" section which have been specified in the article. We have obtained permission from the copyright holder to reproduce figures (or tables) that have previously been published elsewhere.

#### Declarations

#### Competing interests

All authors declare no financial interest competition.

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