Yu *et al.*
Journal of Leather Science and Engineering (2022) 4:11
https://doi.org/10.1186/s42825-022-00085-4

Journal of Leather Science and Engineering

REVIEW

Open Access

Recent strategies of collagen-based biomaterials for cartilage repair: from structure cognition to function endowment

Xiaoyue Yu[1], Haiping Zhang[1], Yiliang Miao[2], Shanbai Xiong[1,3*] and Yang Hu[1,2,3*]

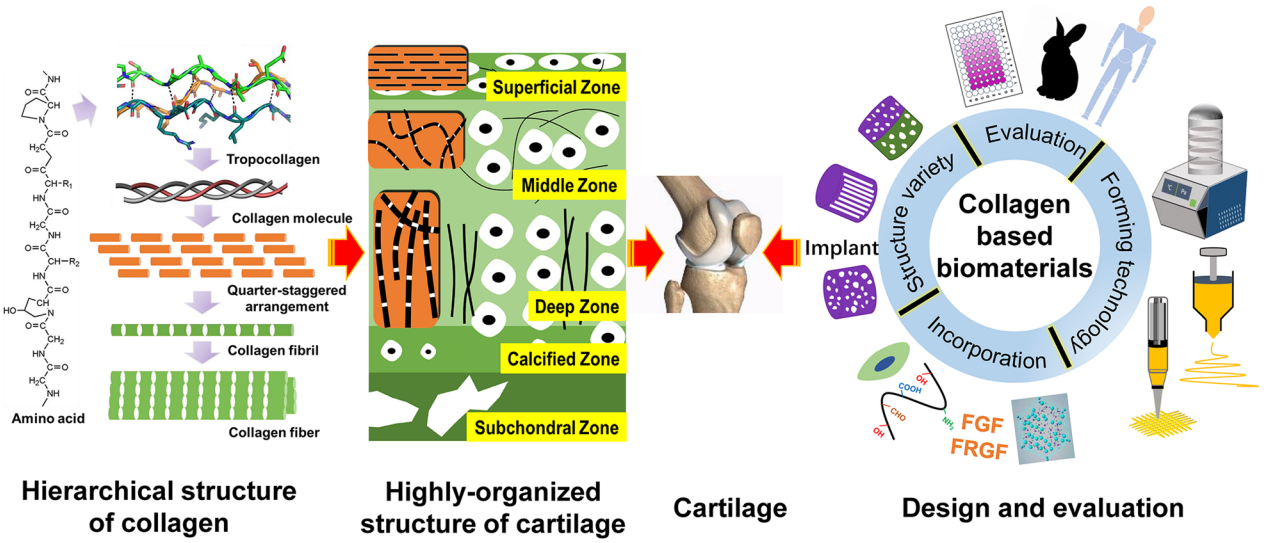
Abstract

Collagen, characteristic in biomimetic composition and hierarchical structure, boasts a huge potential in repairing cartilage defect due to its extraordinary bioactivities and regulated physicochemical properties, such as low immunogenicity, biocompatibility and controllable degradation, which promotes the cell adhesion, migration and proliferation. Therefore, collagen-based biomaterial has been explored as porous scaffolds or functional coatings in cell-free scaffold and tissue engineering strategy for cartilage repairing. Among those forming technologies, freeze-dry is frequently used with special modifications while 3D-printing and electrospinning serve as the structure-controller in a more precise way. Besides, appropriate cross-linking treatment and incorporation with bioactive substance generally help the collagen-based biomaterials to meet the physicochemical requirement in the defect site and strengthen the repairing performance. Furthermore, comprehensive evaluations on the repair effects of biomaterials are sorted out in terms of in vitro, in vivo and clinical assessments, focusing on the morphology observation, characteristic production and critical gene expression. Finally, the challenge of biomaterial-based therapy for cartilage defect repairing was summarized, which is, the adaption to the highly complex structure and functional difference of cartilage.

Keywords: Collagen, Cartilage repair, Hierarchical structure, Structure cognition, Function endowment

*Correspondence: xiongsb@mail.hzau.edu.cn; huyang@mail.hzau.edu.cn
[1] College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, People's Republic of China
Full list of author information is available at the end of the article

Graphical abstract



1 Introduction

Cartilage, which is widely distributed in humans and animals, is composed of dense extracellular matrix (ECM) and sparse chondrocytes. The main chemical components of cartilage are water, collagen and proteoglycans. Highly organized cartilage with a refined structure can meet specific functions, such as bearing mechanical loads, dispersing pressure, and reducing friction. Once a defect or damage occurs, it will also undermine the stability of the tissues connected at both ends of the cartilage. Cartilage is an avascular, aneural and nonlymphatic tissue with limited and scattered chondrocytes, which leads to poor self-healing ability of cartilage defects. Therefore, assistance by exogenous stimulation or biomaterials appears especially important for cartilage repair [1–3]. At present, there are various treatment strategies for cartilage defect repair. Invasive therapies such as microfracture repair, mosaicplasty and cartilage transplantation can control the number of operations, cost and recovery time to a certain extent, but the organization is not well integrated, with many side effects [4]. Scaffold-free therapy, such as injection of stem cells, platelet-rich plasma and growth factors, supplements the defect with bioactive substances that promote cartilage generation and could effectively improve the ability to repair cartilage defects. Nevertheless, the disadvantages lie in the poor physicochemical stability of bioactive substances due to tissue fluid dilution, half-life and immunorejection [5, 6]. Tissue engineering technology combines the advantages of these two types of therapies. This approach employs bioactive scaffolds as a medium to fix chondrocytes or stem cells with chondrogenic potential, as well as bioactive factors that can induce proliferation and differentiation of cells. Tissue engineering scaffolds are very effective in promoting cartilage repair and regeneration [7]. Unfortunately, tissue engineering technology also has limitations. Therefore, therapies such as cell-free scaffolds, acellular cartilage or extracellular matrix implantation, and gene therapy are still receiving considerable attention. These therapies are inseparable from the biomaterial scaffold support [8].

As an implant in cartilage defects, biomaterials need to match the characteristics of natural cartilage tissue in terms of mechanical properties and biodegradability, including good histocompatibility and ability to promote cell proliferation. Overall, collagen is an ideal substrate to prepare cartilage repair scaffolds. Although collagen type II (COL II) is the characteristic component of articular cartilage and showed better chondrogenic ability than COL I [9]. Thus, researchers call for COL II as the substrate of biomaterials for cartilage repair. However, COL II costs higher than COL I. This is mainly due to the wide resource, explicit structure, controllable quality and sufficient study of COL I, while COL II is limited by its resource, distribution and amount in organism, leading to the short cognition of COL II in depth and breadth. Furthermore, the process of isolating and purifying COL II is more complicated than acquiring COL I owing to the higher degree of glycosylation of COL II [10, 11]. Therefore, COL I is superior to COL II in accessibility.

Moreover, both COL I and COL II has been confirmed to maintain the cell phenotype of chondrocytes and support chondrogenic differentiation of human mesenchymal stem cells [12]. In the meanwhile, all the inherent bio-activities of COL I are suitable for developing biomaterials for cartilage repair. For example, considering the positive biocompatibility, biomimetic properties of ECM and wide availability of type I collagen-based materials, collagen type I (COL I) is the preferred repair material. In terms of composition, COL I is one of the main components of the ECM, providing a biomimetic growing environment for implanted cells and demonstrating good promotion of cell adhesion and proliferation. In terms of microstructure control, the oriented arrangement of collagen fibers has been successfully achieved by directional induction technology, thereby mimicking the distribution of collagen fibers in the ECM of cartilage [13]. In terms of biological activity, the excellent biocompatibility and low antigenicity of COL I have already been confirmed [14]. Focusing on the inherent shortcomings of collagen materials, such as inadequate mechanical properties and fast biodegradation, various modification schemes based on physical and chemical methods have been proposed to overcome these issues. In addition, incorporating other bioactive substances can functionalize collagen-based materials while compensating for the inherent defects of collagen [15]. Moreover, noncollagen biomaterials have unique advantages. For example, polycaprolactone (PCL) and polylactic acid have significant mechanical strength, and their characteristics are more suitable for preparing highly precise structures via microfabrication, fiber-based technologies and bioprinting technologies [16, 17]. However, the principal weakness of noncollagen materials lies in the lack of sufficient cell compatibility. Therefore, coating collagen on noncollagen substrates can help strengthen cell adhesion and proliferation [18]. In short, incorporating collagen into implant biomaterials is a smart way to improve the biological activity of implants.

Cartilage repair evaluations of implanted biomaterials can be performed at three levels: in vitro tests, in vivo tests, and clinical trials. In vitro tests aiming to investigate cartilage regeneration start with assessing the cytotoxicity of biomaterials, the ability to promote cell adhesion, proliferation and differentiation, and chondrogenesis-related factors (expression of genes, inflammatory factors, enzymes, key components, etc.) [19, 20]. In vivo tests focus mainly on the ability of implanted materials to assist in repairing cartilage defects; the object of investigation is animals, which have a complete circulatory system. Based on the results of optical observation and immunohistochemistry, the repair or regeneration behavior of cartilage defects is evaluated from the perspectives of integration with the surrounding natural cartilage, the secretion of characteristic substances such as COL and glycosaminoglycan (GAG), and the expression of key factors [21, 22]. Clinical trials are carried out on the basis of in vitro and in vivo optimization, mainly through magnetic resonance imaging (MRI) or computed tomography scanning technology combined with clinical scoring systems to quantify the repair status of patients [23, 24]. Comprehensive and systematic judgments of biomaterials on cartilage repair are made according to these three levels of evaluation.

According to the current reviews that related to the cartilage repair and collagen-based biomaterials, there are few authors introduced the cartilage repair performance of collagen-based biomaterials with respect to structure cognition and function endowment. Meanwhile, there was little summarization concentrated on the design and preparation of articular repair biomaterials, as well as characterization methods of their repairing performance. In detail, reviews related to the collagen-based biomaterials have expanded their biomedical applications in view of the hierarchical structure of collagen and modification methods of collagen-based biomaterials, but the applications were not limited to cartilage repair or cartilage tissue engineering [7, 25]. While reviews concerned about cartilage repairing has collected biomaterials developed from several substrates, not limited to collagen, or mainly emphasized the repairing strategies, or analyzed the chemical structure and physical properties of biomaterials [26–28]. However, reviews focused on the collagen-based biomaterials for cartilage repair are rarely seen, especially associating structural analysis and functional endowment with repairing performance.

Therefore, in this review, we provide a detailed overview about the defects and repair of cartilage and the design and functional evaluation of collagen-based biomaterials, then we summarize the recently developed strategies for cartilage repair as well as design ideas of collagen-based biomaterials. Firstly, the reasons for the occurrence of cartilage defects and the strategy currently implemented for cartilage repair are comprehensively introduced. Then, the objective role of COL I in cartilage repair biomaterials is emphasized, and the forming technology, functional enhancement, and incorporation with bioactive factors are discussed. Subsequently, current indicators related to cartilage repair evaluation are summarized for in vitro, in vivo, and clinical trials. Finally, the challenges and application prospects of collagen-based biomaterials in cartilage repair are put forward. With a view to the design, preparation and functional evaluation of collagen-based cartilage repair biomaterials, this

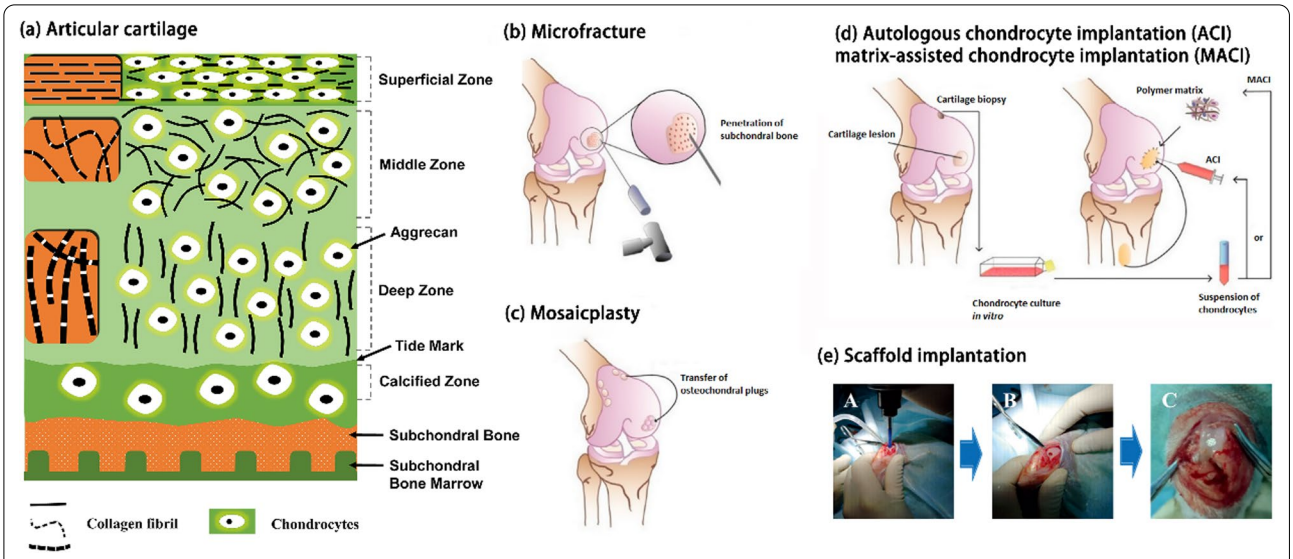


Fig. 1 General structure of cartilage and well-accepted therapy strategies for cartilage defect repair. **a** Diagrammatic representation of highly-organized articular cartilage from human adult with special features. The layered structures are distinguished by chondrocytes morphology, collagen fibrils arrangements, specific biomarkers and characteristic structure are indicated. **b**-**d** Non-biomaterials-based therapy strategies involved in cartilage defect, microfracture, mosaicplasty, autologous chondrocyte (ACI) implantation and matrix-assisted chondrocyte implantation (MACI). Microfracture provides an enriched environment for stimulating tissue regeneration via releasing bone marrow. Mosaicplasty is conducted by patching up the articular cartilage defects with the low weight-bearing areas from articular joint. ACI and MACI are carried out through injecting pre-harvested autologous chondrocytes into the cartilage defect. MACI incorporates chondrocytes into 3D collagen scaffold while ACI performs the in vitro amplification in liquid medium. (Adapted from ref. [3], copyright 2020, with permission from the Elsevier.) **e** Process of biomaterials-based therapy via implantation. (Adapted from ref. [21], copyright 2020, with permission from the Hindawi.)

review aims to provide ideas and clues for the development of preparation technology, the design of repair strategies and the transformation of clinical applications of cartilage repair biomaterials.

2 Structure and defects of cartilage

Cartilage can be subdivided into hyaline cartilage, fibrous cartilage and elastic cartilage according to the types and characteristics of this tissue. Hyaline cartilage, also called articular cartilage, has been widely studied. This review focuses on articular cartilage. Highly organized articular cartilage is mainly composed of loosely arranged chondrocytes and dense extracellular matrix (ECM), with hierarchical structure and characteristic components. While cartilage bears mechanical loads and engages in physiological activities, it inevitably suffers from defects due to mechanical collisions, inflammation or diseases. Lacking blood vessels, nerves and lymphoid tissues, cartilage has poor self-healing ability. Therefore, a variety of repair strategies for cartilage defects have been proposed. For example, surgical treatments through exogenous stimulation, minimally invasive treatments by injecting repairing factors, and tissue engineering therapy by implanting restorative biomaterials all have different advantages and refinement needs. In this section, the composition and structure of cartilage, the etiology of cartilage defects, and the corresponding repair strategies are described in detail.

2.1 The composition of articular cartilage

Cartilage is composed primarily of chondrocytes and ECM (Fig. 1a). Chondrocytes account for approximately 2% of the total volume of articular cartilage and are in charge of the production, secretion and maintenance of ECM. Chondrocytes originate from mesenchymal stem cells (MSCs) and are mature and highly differentiated cells. In the superficial zone of articular cartilage, chondrocytes are mostly distributed in a single, fusiform shape and secrete large amounts of chondroitin sulfate. In the middle zone of cartilage, chondrocytes exist as an isogenous group with a spherical shape (2–8 cells) [29]. The metabolic activity of chondrocytes is changed by responding to surrounding stimuli, such as growth factors, mechanical loads, piezoelectric force, and hydrostatic pressure. Due to their limited replication potential, the inherent healing ability of chondrocytes is insignificant when suffering from defects [30]. The ECM of cartilage mainly contains water, collagen and proteoglycans. As a critical component of ECM, water accounts for approximately 80% of the wet weight of ECM and helps provide lubrication and transport nutrients [1]. Collagen

(COL) is another important component of the ECM, accounting for approximately 60% of the dry weight of cartilage. Collagen type II (COL II) in articular cartilage accounts for approximately 90–95% of the extracellular matrix collagen. The characteristic triple helix structure of COL maintains the mechanical homeostasis of chondrocytes and ECM by providing fundamental anti-shear and tensile properties [7]. Proteoglycan accounts for 10–15% of the wet weight of ECM in articular cartilage; it is a highly glycosylated protein monomer secreted by chondrocytes that covalently binds to a core protein and single/multiple linear glycosaminoglycan chains.

Moreover, the ECM has three clear gradient structures according to the distance from chondrocytes (Fig. 1a) and characteristic compositions. (1) Pericellular region: locate at the surrounding cell membrane within 2 μm. Be characteristic in collagen type VI. Mainly consist of proteoglycans, glycoproteins and noncollagen components. (2) Be rich in chondroitin sulfate and proteoglycan. The collagen fibers are formed as a net-like structure. (3) Interterritorial region: Be rich in keratin sulfate. Own the thickest collagen fibers, which are arranged randomly [7, 30].

2.2 Hierarchical structure of articular cartilage

Articular cartilage has a thickness of approximately 2–4 mm and four levels of structure that are highly differentiated (Fig. 1a) [29]. According to the arrangement direction of COL II fibers and the content of proteoglycan, the articular cartilage is divided into the following four levels. (1) The superficial zone is the part where articular cartilage and synovial fluid come in contact, accounting for approximately 10–20% of the cartilage thickness. It contains a large number of flat chondrocytes and a small amount of proteoglycans. Densely distributed COL II and collagen type VI fibers form thin layers in the ECM, which are organized parallel to the articular surface and show significant stretching strength. (2) The middle zone of the COL fiber network has a larger diameter and is organized in a random manner, enveloping spherical chondrocytes. The proteoglycan content is greater than that in the superficial zone. The middle zone displays a certain degree of stress resistance. (3) The deep zone accounts for approximately 30% of the cartilage volume. The chondrocytes are parallel to collagen fibers. COL fibers here have diameters of approximately 70–120 nm and are arranged radially perpendicular to the articular surface. These fibers show the greatest pressure resistance in articular cartilage. Additionally, the deep zone has the highest proteoglycan content and the lowest water content. (4) The calcified zone, also called calcified cartilage, represents the transition area from cartilage to subchondral bone. The scarce chondrocytes in this zone are hypertrophic [3, 7, 30].

2.3 The etiology and degree of cartilage defect

The normal metabolism of chondrocytes is regulated by mechanical, environmental and genetic factors. Regular joint movement and moderate dynamic load are necessary factors to maintain the stability of chondrocytes. The etiology of cartilage defects includes but is not limited to wear, trauma, and degenerative diseases. Accidental trauma and inappropriate high-intensity exercise can cause acute defects in cartilage, such as fractures and meniscus tears. Because chondrocytes are extremely sensitive to mechanical stimuli such as external dynamic compression, fluid shear, tissue shear, and hydrostatic pressure, excessive mechanical stimuli disrupt the physiological balance of chondrocytes and then induce cartilage-related diseases. First, the death of chondrocytes and rupture of the collagen fiber network are attributed to impacting cartilage with a load of 15–20 MPa, which changes the metabolic behavior of the tissue and the water content of the ECM, resulting in permanent defects in the cartilage ECM [31]. In addition, due to the effects of obesity and occupation, chronic wear and degradation of cartilage tissue are usually caused by long-term and abnormal cumulative loads, leading to an imbalance in the catabolism and anabolism of the ECM [32]. Most cartilage defects evolve into osteoarthritis. Under the abnormal expression or appearance of matrix-degrading protease, inflammatory factors and chemokines or other biochemical stimulations, chondrocytes convert from a resting state to a developing state, manifesting by proliferation and hypertrophy and overexpression of ECM-related proteins and matrix-degrading enzymes. Accordingly, remodeling and cartilage calcification of the ECM are triggered, which can aggravate the disease while destroying chondrocytes [33, 34].

According to the classification system provided by the International Cartilage Repair Society (ICRS), cartilage defects can be divided into four levels. From degree I to degree IV, the severity of the defect grows [17].

2.4 Current repair strategies

Once the area or thickness of the cartilage defect exceeds the critical size (4 mm), it is difficult for cartilage to undergo self-repair [35]. With continuous exploration and development, further understanding and breakthroughs in cartilage repair strategies and techniques have been put forward.

Strategies of bone marrow stimulation (BMS) and autogenous cartilage transplantation, such as arthroscopic minimally invasive treatment, microfracture, and mosaicplasty, can regulate the frequency of operations, costs and recovery time and are more suitable for small-scale defects (Fig. 1b,c). However, these kinds of therapies generally transplant non-load-bearing bone in the defect

area. Nevertheless, the area that can be transplanted is quite limited. Additionally, unmatched mechanical properties lead to unsatisfactory integration of the tissue, and it is difficult to regenerate the same type of cartilage. In addition, factors such as patient age, disease risk, and persistent postoperative pain affect the repair performance [4]. Although the developed allograft strategy solves the problem of donor limitations and can initially treat large cartilage defects to a certain extent [36], a perfect match between the allograft and the natural structure is still hard to realize, which leads to an imbalance in biomechanical load and then can aggravate degenerative diseases [5]. For patients with large cartilage defects and microfractures, autologous/allogeneic chondrocyte implantation (ACI) or matrix-induced autologous chondrocyte implantation (MACI) is another repair strategy (Fig. 2d). This technique involves two surgical operations. In the first operation, chondrocytes are isolated from healthy articular cartilage, amplified in vitro, and then seeded on a scaffold. In the second operation, the scaffold with cells is implanted into cartilage defects [37, 38]. Although BMS and ACI/MACI show good repair performance in terms of the clinical symptoms and activities of patients, problems such as operation cost, regulatory restrictions, and time for chondrocyte proliferation need further consideration. In the process of in vitro amplification, autologous chondrocytes easily dedifferentiate into fibrous cells and then produce other types of cartilage tissue at the repair site [39, 40]. Therefore, mesenchymal stem cells (MSCs) have been chosen to replace autologous chondrocytes as an improved strategy owing to their potential for cartilage differentiation and great self-renewal ability. MSCs play a critical role in regulating cell survival and tissue repair, as well as the immune-regulatory effect by reducing inflammation. For example, exosomes (Exos) secreted by bone marrow mesenchymal stem cells (BMSCs) have been shown to inhibit the expression of proinflammatory factors, prevent the apoptosis of chondrocytes induced by lipopolysaccharide, and promote the proliferation of chondrocytes, which effectively alleviates the aggravation of osteoarthritis [34, 41].

Cell-free scaffold strategies focus on the selection and design of biomaterials. The biocompatibility and degradability of the biomaterials need to be considered. Refined structures should also be designed for the diffusion and exchange of nutrients and metabolites. The most important aspect is to adapt to the tissue and meet the requirements for anisotropic mechanical behavior [7]. Delivery scaffolds incorporated with proteins, nucleic acids and growth factors can facilitate the directional migration of endogenous host cells to defect sites, strengthen the secretion of cartilage ECM, and enhance the repairing ability of acellular scaffolds (Fig. 1e). Employing decellularized cartilage or cartilage ECM as a biological scaffold is another advanced repair strategy that directly provides an absolutely natural environment where the chondrocytes in the ECM are located without further functionalization of the biomaterial [42, 43]. Lim et al. found that the cranial cartilage of squid (*Dosidicus gigas*) is relatively loose, which is suitable for cell-free therapy as a tissue engineering scaffold. Decellularized cranial cartilage retained the original microstructure of the ECM and was interconnected with porous structures. Chondrocytes had good activity and migration in the scaffold. The cartilage scaffold obviously promoted cartilage regeneration [44]. As another advancement in cell-free scaffold strategies, tissue engineering technology combines natural, synthetic or mixed biological materials and/or growth factors and/or cells. In tissue engineering strategies, biological materials are not only used to support cell proliferation, differentiation and migration but also used as carriers of bioactive factors to induce and strengthen the functions of chondrocytes, which is a promising repair strategy in the field of cartilage repair [7, 45]. Whether cell-free scaffolds or tissue engineering scaffolds are used, it is important to adjust the preparation technology to present a layered and porous structure of scaffolds in the spatial dimension and achieve the required mechanical properties and mass transfer capabilities. Therefore, scaffolds are more favorable for cells to realize gradient distribution and functional play by providing a biomimetic environment. Finally, the scaffold promotes tissue integration and cartilage defect repair [46, 47].

In addition to the mainstream scaffold repair strategies, gene therapy is another effective method. Using biomaterials for gene delivery can avoid dilution by synovial fluid [48]. Furthermore, to better simulate the biomechanical behavior of cartilage for cell proliferation and differentiation, appropriate auxiliary means such as hypoxia, hydrostatic pressure, low-intensity laser and compressive force can be applied on the basis of implanting biomaterials, showing better effects in promoting cartilage repair [49–52].

3 Structure and function of collagen

As an abundant component in mammals, collagen (COL) is widely found in soft and hard tissues, such as tendons, skin, cornea, bones and teeth, and accounts for approximately 30% of the total protein in mammals. Collagen not only establishes tissue organization itself but also functions through biomineralization or combining with minerals to form nanocomposites [53, 54]. Based on the advantages of wide resources, excellent biological activity, and strong plasticity of COL I, various sizes and shapes of bioscaffolds, such as hydrogels, sponges, and fiber mats, have been developed for implanted tissue repair [57].

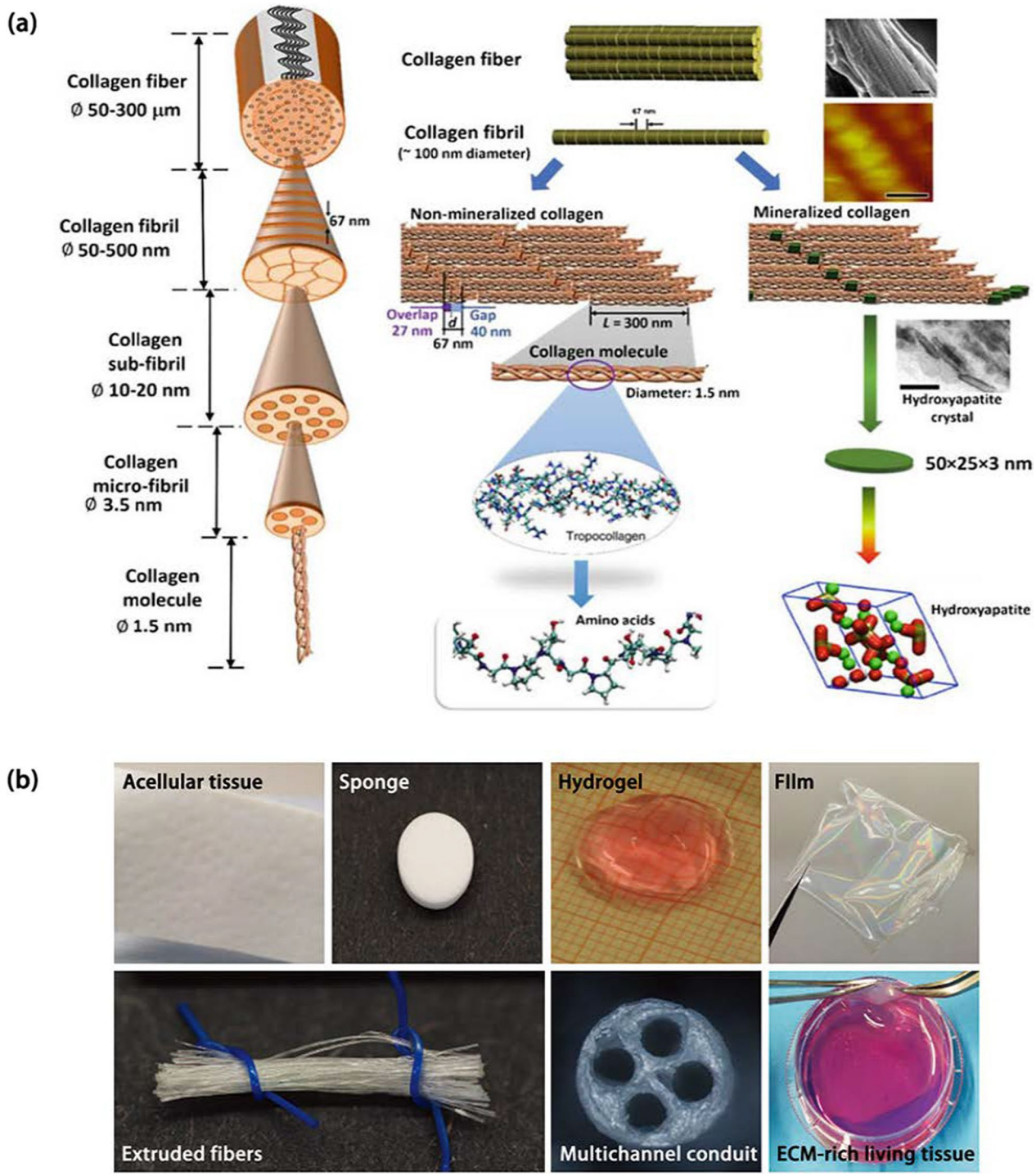


Fig. 2 **a** Hierarchical structure of collagen fibers from nanoscale to micrometer scale. (Adapted from ref. [54], copyright 2021, with permission from Springer Nature.) **b** Various forms of collagen-based biomaterials. (Adapted from ref. [62], copyright 2019, with permission from Wiley.)

3.1 Hierarchical structure of COL I

In biological tissues, collagen has a refined and complex multilayered structure (Fig. 2a). COL I is described herein as a characteristic example. In the primary structure, the repetitive amino acid sequence of COL I is composed of (glycine-X–Y)n, and X and Y usually represent proline and hydroxyproline or any amino acids, except glycine. Hydroxyproline is a characteristic amino acid of collagen and can provide binding sites for water molecules to form hydrogen bonds, which play a crucial role in maintaining the stability of the triple helix structure. Hydroxyproline and hydroxylysine are associated with the formation of hydrogen bonds and van der Waals forces between polypeptide chains [60, 61]. In the secondary structure, every three polypeptide chains form one left-handed α-helix by crimping in the center and form a polyproline II helical structure under the electrostatic repulsion between proline and hydroxyproline and the hydrogen bonding between each amino acid residue [54]. On the basis of the secondary structure, the left-hand α-helix structure is further crimped and folded to form a right-handed α-helix conformation, the tertiary structure, called the procollagen molecule. Its average molecular weight is 300 kDa, its length is approximately 300 nm, and its diameter is approximately 1.5 nm. The hydrogen bond and covalent interaction between the NH group of glycine and the adjacent O = C(X) determine the stability of the conformation [55]. Subsequently, the procollagen molecules aggregate in the horizontal and vertical directions to form the quaternary structure. In the horizontal direction, procollagen is arranged in a straight line with a spacing of 64–67 nm (D units). In the longitudinal direction, the collagen microfibrils are arranged in parallel with a spacing of 40 nm. At this time, intermolecular and intramolecular cross-links have been formed. Then, the collagen microfibrils are stretched laterally in a quarter staggered arrangement and assembled into collagen fibrils with an ordered crystal structure. The fibrils further aggregate and line up into bundles to form collagen fibers. Finally, the collagen fibers are entangled with each other to construct a three-dimensional network structure, establishing the basic structure of the tissue. In addition, collagen fibers demonstrate varied directional arrangement in specific organs and tissues [53, 62].

3.2 The role of COL I in cartilage repair

Since Ehrmann et al. reported that collagen gel promoted cell growth in vitro and affected the morphology, migration, adhesion and even differentiation behavior of cells [63], researchers have gradually realized the positive effects of COL on cell development and its potential application in cartilage repair. At present, a number of studies have confirmed that collagen-based biomaterials have excellent biological activities, such as the biocompatibility of collagen itself, the controllable biodegradation rate through cross-linking modification, low immunogenicity and weak antigenicity, the ability for host cells to adhere and migrate, and the adjusted degree of calcification. It is worth noting that the COL I hydrogel can effectively assist cartilage repair and maintain the regenerated cartilage type as hyaline cartilage [64].

The complex and highly organized hierarchical structure of COL makes collagen-based biomaterials have a certain tensile strength [65], but their weak mechanical properties at the macro level cannot meet the mechanical requirements of natural cartilage tissue; hence, physical incorporation or chemical modification is needed. The reason why COL I affects cell behavior lies in the abundant bioactive sites on its triple helix structure, which can bind bioactive molecules or interact with receptors on the cell surface to trigger biological events or regulate the metabolic behavior of cells. For example, peptides such as RGD (Arg-Gly-Asp) and DGEA (Asp-Gly-Glu-Ala) support cell attachment and growth. Therefore, the implantation and degradation of collagen-based bioscaffolds in vivo are beneficial to the growth and metabolic behavior of host cells, but COL I itself does not show osteoinductive effects [66, 67].

In addition, cells adhering to COL secrete specific enzymes to degrade COL and secrete synthesized collagen to the outside of the cell, which creates a dynamic balance of 'removal-remodeling-replacement' of extracellular COL [65]. The physiological environment of the implantation site, such as the presence of collagenase and phagocytosis, affects the degradation behavior of collagen-based biomaterials. Appropriate modification can decrease the degradation rate of implanted COL in tissues. The low immunogenicity of COL I lies in pepsin digestion when extracting COL, which can hydrolyze the antigenic determinants of COL on the telopeptide, minimizing the immunogenicity of the COL substrate. The biomimetic calcified layer of natural cartilage can be well constructed through the accumulation and mineralization of calcium ions and phosphate ions in the gaps of collagen fibers, which fully provides a simulation system for host chondrocytes. Moreover, the biomaterials used to repair cartilage must strictly control the degree of calcification and cross-linking so that the degradation rate and repair ability of implanted COL can be appropriately regulated [14]. These properties described above endow COL I with wide application prospects as a biomaterial in tissue engineering, whether used alone or as a composite material [15, 68].

Furthermore, other collagen components play a role in the process of cartilage repair. For instance, collagen type VI is mainly located in the pericellular matrix of

developing and mature chondrocytes and is indispensable in regulating the swelling of chondrocytes and maintaining the biomechanical integrity of the pericellular matrix [69]. Collagen type III is the third most important collagen in cartilage, participating in the repair of matrix damage, and is abundant in damaged tissues during repair. The deposition of collagen type III occurs in the articular cartilage of adults, but it is dominant in osteoarthritis patients. Therefore, collagen type III is considered to be a potential biomarker in matrix repair or pathophysiological diagnosis of cartilage [70–72].

4 Design and preparation of collagen-based biomaterials for cartilage defect repair

There are various forms of existing collagen-based biomaterials for cartilage defect repair, including hydrogels, sponges, films, fiber mats, membranes, etc. (Fig. 2b). The form is determined by the difference in the repaired zones, preparation methods and functions. Considering the metabolic environment in the interzone during cartilage development, a porous three-dimensional scaffold is preferred to support and maintain the phenotype of cartilage. Hydrogels have become competitive products because of their ability to reproduce the solid-to-liquid ratio of cartilage tissue, imitate the structure of native cartilage, and achieve uniform encapsulation and diffusion of chondrocytes [45]. In view of the problems that pristine collagen cannot fully meet the physical and chemical characteristics of natural cartilage tissue and cannot precisely control the differentiation of cells, as well as the type of regenerated cartilage after cell implantation, researchers have developed a series of collagen-based biomaterials with high-quality cartilage repair capabilities by adopting appropriate preparation technologies, designing ingenious physicochemical cross-linking methods and incorporating bioactive factors. Herein, we provide an overview about the forming technology and cross-linking methods for designing collagen-based biomaterials, as well as the incorporated functional substances.

4.1 Forming and cross-linking of collagen-based biomaterials

4.1.1 Forming technology

The self-assembly behavior of COL in physiological environments involves gelatinization of COL according to its characteristics so that the injectable hydrogel can be prepared. Owing to the less challenging self-assembly technology in vivo, cells and functional factors are easily pre-encapsulated in COL solution to construct injectable COL hydrogels for tissue engineering [73, 74]. In the same way, COL hydrogels have been developed in vitro under neutral conditions with specific shapes [75].

Freeze-drying technology, another general forming technology, is mainly applied to prepare porous collagen scaffolds. Tang et al. reported that collagen scaffolds with elasticity and shear were prepared by pouring acidic collagen into a mold and freeze-drying at -80 ℃, and the porous scaffold could also absorb solutions containing bioactive factors [76]. Freeze-drying technology is conducted at low temperatures with no requirement for pore-forming agents, which retain the complete structure and bioactivity of COL molecules. The pore size and morphology of the COL scaffold after freeze-drying can be controlled by adjusting the processing parameters, such as freezing rate and temperature, as well as the concentration of COL. In fact, porous scaffolds prepared by freeze-drying technology often show irregular structures and varied pore sizes (15–35 μm) with little gradient difference in microscopic morphology [77]. Therefore, a variety of preparation techniques based on freeze-drying have been developed to prepare collagen scaffolds with refined structures. The method of applying a freezing cycle combined with freeze-drying was presented by Amann et al. The authors constructed a three-layered, porous COL I scaffold with gradient difference to simulate the tissues and interfaces of cartilage. Stem cells were successfully differentiated in specific regions induced by the scaffold [78]. A porous hydrogel scaffold consisting of COL II and polyvinyl alcohol was prepared by Lan et al. by combining freeze–thaw cycles and freeze-drying, which had good binding strength with the interface of cartilage tissue (Fig. 3c) [79]. Interestingly, ice particles with fixed sizes were employed as pore-forming agents to mix with the COL solution (Fig. 3a). After freeze-drying, the porous COL scaffold showed interconnected pores (both with large and small particles) and a porosity higher than 98% [80]. It is worth noting that oriented COL scaffolds could also be prepared by adjusting the freezing parameters. Qi et al. used sequential and unidirectional freezing methods to regulate the orientation of COL fibers (Fig. 3d). The specific method involved placing the mold injected with COL solution on a metal plate at − 20 °C for 30 min at a certain speed and then freeze-drying it at − 80 °C. Scanning electron microscopy images illustrated the parallel arranged and connected microtubules inside the COL scaffold, the micromorphology of which promoted the regeneration of hyaline cartilage induced by BMSCs [81]. Wang et al. confirmed again that a one-direction freezing method could be combined with freeze-drying technology to prepare COL scaffolds with a directional porous structure, which is conducive for BMSC proliferation and adhesion [13]. Feng et al. obtained collagen scaffolds with different pore types after freeze-drying.

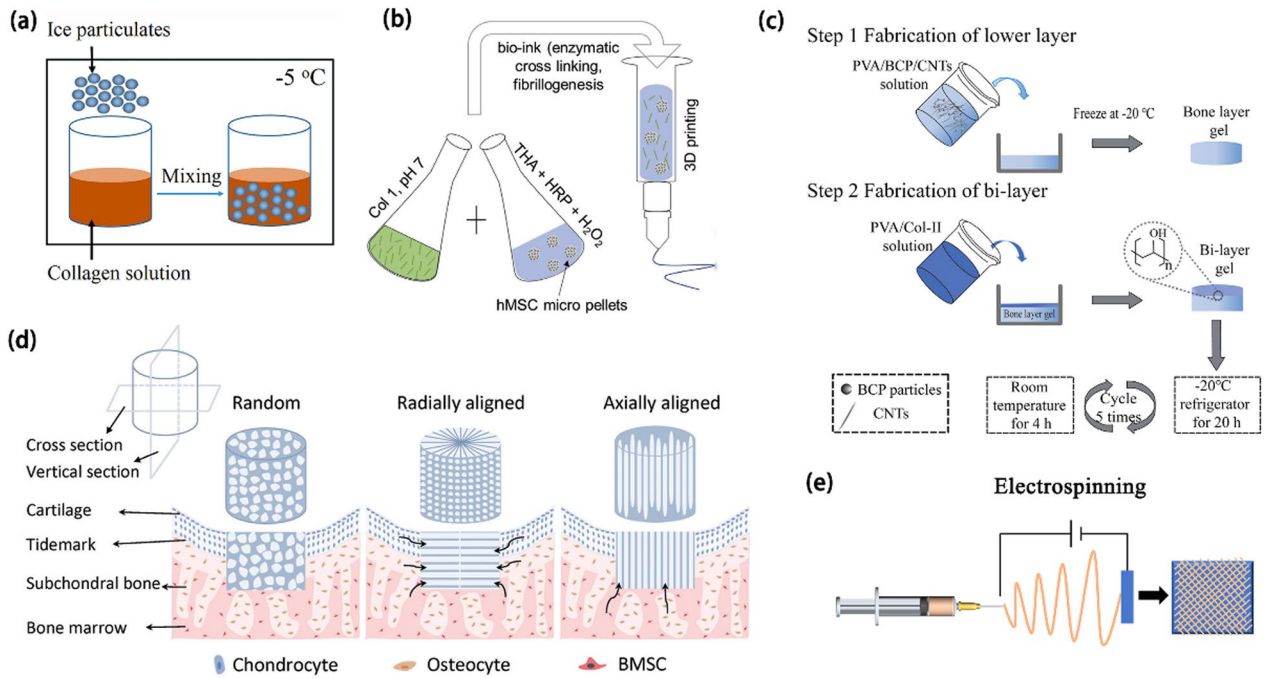


Fig. 3 Novel forming technologies of collagen-based biomaterials. **a** Ice particle for pore-forming. Ice particle in specific size was used as a pore-forming agent to develop collagen scaffold with controlled pore structure before freeze-drying. (Adapted from ref. [80], copyright 2020, with permission from Elsevier.) **b** Three-dimensional (3D) printing forming technology. 3D printing was employed to fabricate bio-matrix containing collagen type I (COL I), tyramine derivative of hyaluronan (THA) and human bone marrow–derived mesenchymal stromal cell (hMSC), while horseradish peroxidase was the cross-linking agent for THA. (Adapted from ref. [84], copyright 2020, with permission from Elsevier.) **c** Combination of sequential freeze, freeze thawing and freeze-drying to prepare bilayer scaffold. PVA, BCP, CNTS and Col II represent Polyvinyl alcohol, biphasic calcium phosphate, carbon nanotubes and collagen type II, respectively. (Adapted from ref. [79], copyright 2021, with permission from Elsevier.) **d** The oriented architecture of collagen-based scaffolds by sequential unidirectional freezing method with random pores, radially aligned pores and axially aligned pores. (Adapted from ref. [82], copyright 2020, with permission from Royal Society of Chemistry.) **e** Collagen-based nanofibrous membrane formed by electrospinning

The authors prepared frozen objects with different orientations of ice crystals by controlling the material and size of the mold, as well as the freezing temperature and time [82]. In addition, liquid casting combined with freeze-drying showed stable formation of COL in the mold. She et al. developed a 3D printed composite scaffold by casting collagen liquid into the hollow frame of polycaprolactone (PCL), followed by freeze-drying forming (Fig. 3b) [83]. Although freeze-drying technology has been gradually improved, it is still difficult to create complex geometries or control cell distribution in the scaffold. Exogenous technologies are urgently needed for freeze-drying to enhance the functionality and biomimetic properties of freeze-dried scaffolds.

The development and application of new technologies have created more possibilities to prepare collagen-based biomaterials for cartilage repair. New biomanufacturing technologies represented by 3D printing have achieved precise control of the scaffold structure. Multilayered scaffolds with controllable biodegradability, pore structure and mechanical properties have been achieved by the bottom-up printing method. The rheological properties of COL I solution were previously evaluated by Lee et al. Good 3D printing adaptability was demonstrated by having a higher storage modulus than loss modulus, and the composite viscosity of COL decreased with increasing shear frequency [19]. In addition, hyaluronic acid improved the viscoelasticity of the composite solution while assisting in the uniform distribution of collagen. Therefore, printed COL scaffolds showed oriented and anisotropic collagen fibers, which provide a better biomimetic simulation of ECM [84]. However, because the interlayer adhesion of scaffolds is strengthened by 3D printing technology, the shrinkage and swelling of the scaffolds are limited. Thus, 3D printing cannot be satisfied by microdesign of the injectable scaffold [85]. Electrospinning technology is also widely used to prepare highly porous scaffolds for tissue repair and regeneration, which show superiority in terms of a high specific surface area, good permeability, adjustable degradation rate, and local sustained release of drugs (Fig. 3e) [77]. For example, collagen-PCL nanofiber membranes were

fabricated by electrospinning technology, and the transport of nutrients was confirmed to be not hindered [86]. The combination of electrohydrodynamic-direct printing with wet spinning was used to fabricate a multilayer fibrous collagen mesh scaffold, which exhibited flexibility and recoverable elasticity [85]. The limitations of traditional technologies are being overcome by emerging technologies. Specifically, mechanical properties, structural details, microarchitectures such as the size, geometry and interconnection of pores, and cell distribution of complex engineering scaffolds can be simultaneously and accurately controlled [16]. Although these new technologies are rarely used in preparing collagen-based scaffolds or cartilage defect repair, the advantages of these emerging technologies are worthy of reference and learning.

4.1.2 Cross-linking method

To meet the demand for imitating the physicochemical environment of cartilage, it is necessary to improve the poor mechanical properties, high swelling rate, and low enzymatic resistance of pristine COL substrates. Therefore, when designing biomaterials, the integration of forming technology and cross-linking methods can assist in the formation of COL and strengthen the formed biomaterials. We have previously overviewed the physical and chemical modifications of COL at length [87]. Nevertheless, alternative cross-linking methods for collagen-based biomaterials are limited, attributed to additional complex components such as bioactive compounds, active factors and cells. Therefore, the adaptability and side effects of added components are worth considering, as is their influence on collagen-based biomaterials regarding inflammation and tissue integration ability after implantation in vivo.

Recently, collagen-based cross-linking methods for cartilage repair have adopted conservative and low-toxicity cross-linking agents, such as genipin [75], oxidized gum arabic [22], N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide/N-hydroxysuccinimide (EDC/NHS) [88], tannic acid [89], aldehyde glycogen (Fig. 4a) [90], enzymes [91], and 1,4-butanediol diglycidyl ether (BDDGE) [20]. Zhou et al. prepared a collagen-chondroitin sulfate/collagen-nanohydroxyapatite bilayer scaffold by combining freeze-drying and EDC/NHS cross-linking. The simultaneous regeneration of cartilage and subchondral bone was confirmed in animal models (Fig. 4c) [60]. Freeze-dried COL-GAG composite scaffolds were prepared by combining dehydrothermal treatment with EDC/NHS cross-linking, and fibroblasts loaded in the scaffold behaved as lubrication [92]. After modification by 1,4-butanediol diglycidyl ether (BDDGE) and sodium carbonate, the pristine COL I scaffold exhibited proper biocompatibility with injured cartilage tissue. Host cells were effectively recruited and then differentiated into cartilage, which promoted the development of new cartilage-like tissue [93]. BDDGE was also applied to fabricate biomimetic scaffolds containing COL and chondroitin sulfate. Cell-free scaffolds promote the regeneration of cartilage by regulating the immune environment and effectively slow the chronic inflammatory response associated with cartilage defects [20]. By forming Schiff base bonds and electrostatic interactions with COL and hydroxyapatite, respectively, the aldehyde glycogen was used to stabilize a COL-hydroxyapatite composite hydrogel [90]. In addition, hydrogen peroxide and horseradish peroxidase were used as cross-linking agents to prepare injectable composite hydrogels containing collagen and hydroxy-phenyl-propionic acid-functioning gelatin. The stiffness of the hydrogel could be adjusted by varying the concentrations of hydrogen peroxide within noncytotoxic levels [91].

Furthermore, cross-linkers were observed not only to strengthen the intermolecular cross-linking among COL molecules but also to stably bind active ingredients to COL. Zheng et al. proposed cadmium selenide quantum dots (QDs) as fillers to prepare injectable COL hydrogels by genipin cross-linking, which demonstrated both enhanced stiffness of the COL hydrogels and specific differentiation of BMSCs into chondrocytes. In addition, the QDs acted as photosensitizers to produce reactive oxygen species (ROS), which further synergistically promoted cartilage regeneration [94]. Another similar report used carbon dot nanoparticles bound to COL via genipin cross-linking, which improved the mechanical properties of the COL substrate. Photodynamic therapy was also used to produce ROS, which increased the differentiation degree of BMSCs to chondrocytes by approximately 50%. At the same time, the expression of specific cartilage-related genes was doubled, thereby promoting the secretion of GAG and ultimately accelerating cartilage regeneration [95].

It is worth noting that macrophages are the first responders to implant biomaterials. Their polarization to proinflammatory or anti-inflammatory states determines the success or failure of implantation. Sridharan et al. mentioned that the dependency of macrophages on COL scaffolds relied on the cross-linking agents used, so comprehensive consideration of the clinical suitability and alternatives of cross-linkers is necessary for individual treatment [96]. Although most of the literature confirmed positive availability of fabricated COL scaffolds, there were still some COL scaffolds failed to show the promoting effects on cartilage regeneration [97]. As we emphasized here, the selection of forming technology and cross-linking method must be comprehensively considered in terms of the processing adaptability of

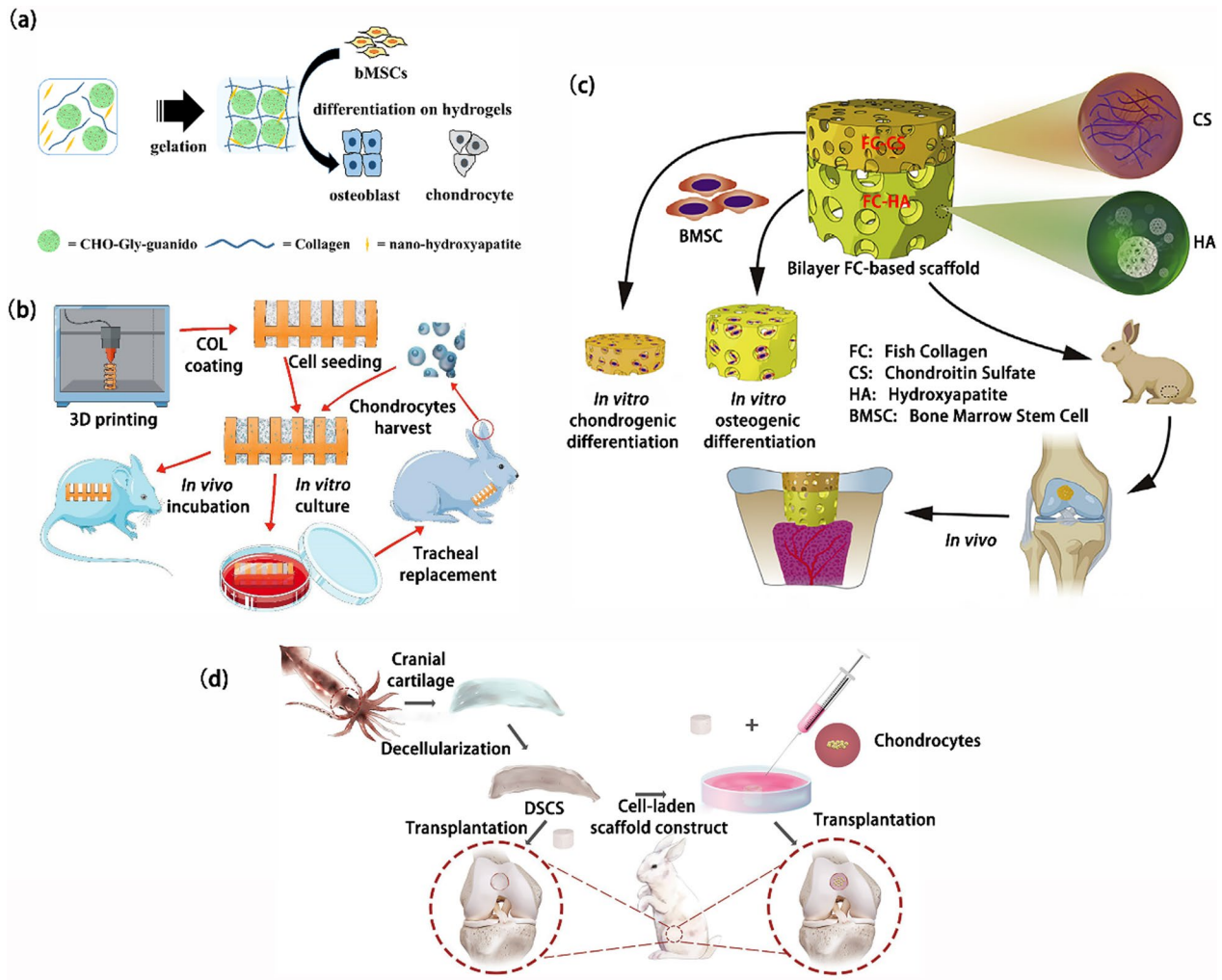


Fig. 4 Incorporation and application of collagen-based biomaterials for cartilage repair. **a** Collagen-nanohydroxyapatite composite hydrogel fabricated by aldehyde glycogen cross-linking. The hydrogel induced bone mesenchymal stem cells (BMSCs) differentiate into osteoblasts or chondrocytes. (Adapted from ref. [90], copyright 2020, with permission from ACS Publications.) **b** Collagen embedded scaffold fabricated with 3D printing. The biomimetic scaffold recolonized by chondrocytes elicited a satisfactory repair outcome for cartilage replacement both in vitro and in vivo. (Adapted from ref. [83], copyright 2021, with permission from Frontiers Media S.A.) **c** Bilayer collagen-based scaffold with varied pore sizes and crucial components. The bottom layer with larger pores and hydroxyapatite as well as the top layer with small pores and chondroitin sulfate were established to mimic the morphology of cartilage and bone tissue. (Adapted from ref. [60], copyright 2020, with permission from Elsevier.) **d** Decellularized cartilage tissue laden with chondrocytes as a biomimetic implantation. Cartilage tissue maintained critical structure and component of the extracellular matrix to the maximum extent. (Adapted from ref. [44], copyright 2020, with permission from the Royal Society of Chemistry.)

raw materials, the impact of cross-linking methods on the composite components, and the potential risks after implantation in vivo.

4.2 Incorporated fabrication of collagen-based biomaterials for cartilage repair

According to clinical follow-up regarding articular cartilage defect repair, the comprehensive repair ability of cell-free COL I scaffolds is not ideal, especially for larger defects. Pristine COL scaffolds are not capable of fully supporting the repair and regeneration of cartilage [98]. Therefore, a series of designs of functional COL scaffolds have been proposed, focusing on incorporating cells and bioactive substances on scaffolds to strengthen the repair and regeneration ability of the COL scaffold [99].

4.2.1 Incorporating bioactive substances

Bioactive substances mainly include naturally derived/synthesized organics, bioactive inorganics, small

functional molecules and genes. Designs suitable for cartilage repair rely on their multifunctional bioactivities.

Natural macromolecules such as hyaluronic acid, chitosan, alginate, ECM, and acellular cartilage tissue effectively enhance the physicochemical properties and cartilage repair capabilities of collagen-based biomaterials. For example, hyaluronic acid is conducive to the production and maintenance of ECM secreted from chondrocytes, with strong water-retention ability [58]. Chitosan is known to have a structure similar to that of GAG and can form a porous structure with certain antibacterial properties, low toxicity, biocompatibility, and osteoconductivity, the low degradation rate of which can be compensated by COL [100, 101]. The elasticity of COL substrates was also shown to be enhanced by cross-linking between alginate and Ca^{2+} [102]. More importantly, the native extracellular microenvironment that regulates cell proliferation and differentiation can be simulated by ECM. Wang et al. evaluated the ECM-collagen composite hydrogel of articular cartilage in newborn, juvenile and adult rabbits. Regarding the effects of fabricated hydrogels for inducing BMSCs to differentiate into chondrocytes, a comparison suggested that the ECM derived from juvenile rabbits was good at promoting cartilage formation and preventing matrix calcification [103]. Huang et al. developed a three-layer scaffold using COL II as a scaffold, loaded with porcine autologous BMSCs covalently connected with decellularized calcified cartilage (Fig. 4d). With the help of decellularized calcified cartilage, the incorporated scaffold mainly induced the regeneration of hyaline cartilage at the defect, but the defects in the noncalcified cartilage group were filled with fibrocartilage [104].

Based on their similarity to native cartilage, ceramic inorganics are also used to repair cartilage defects. It has been confirmed that hydroxyapatite has definite biological activity, osteoconductivity and biodegradability. The combination of COL and hydroxyapatite showed synergistic performance in enhancing osteoconductivity [56]. In addition, hydroxyapatite improved the physicochemical properties and biological activities of the collagen sponge. There are various designs for collagen-hydroxyapatite composite scaffolds. To enhance the mechanical properties of the composite scaffold, Crovace et al. proposed a 'honeycomb' structure by embedding hydroxyapatite in a porous collagen scaffold with a columnar shape. Animal experiments indicated the promising repair effect of the embedding model [46]. Biphasic calcium phosphate also has good osteoinductivity. Cai et al. constructed a double-layer scaffold with COL I hydrogel and biphasic calcium phosphate as the biomimetic cartilage layer and subchondral bone, respectively. The characteristic structure provided a suitable location for cell migration, proliferation and secretion, which effectively promoted the regeneration of cartilage and subchondral bone. The newly regenerated cartilage was similar to native cartilage with respect to structure and thickness, including seamless integration with the surrounding cartilage [105]. The application of metal in cartilage repair is still under development. Based on the good mechanical properties of porous tantalum and the ability to induce osteogenic differentiation of stem cells, the incorporation of a three-dimensional COL membrane with porous tantalum exhibited committed differentiation of loaded stem cells, maintained the phenotype of chondrocytes and highly expressed genes related to cartilage production. The incorporated membrane showed significant therapeutic performance in repairing large osteochondral defects in goats [106].

'Growth factors' is a collective term for a class of bioactive peptides. These factors regulate the local microenvironment of joints by stimulating the anabolism and catabolism of cells, which promotes the growth of chondrocytes and cartilage production and assists in the repair of larger cartilage defects. Several essential growth factors are well confirmed, such as the transforming growth factor superfamily, fibroblast growth factor family, insulin-like growth factor, and platelet-derived growth factor [107]. Song et al. reported that supplementing fibroblast factor 2 reversed catabolism to anabolic metabolism under cartilage damage, which was helpful to stabilize the ECM and promote cartilage regeneration [108]. Supported and delivered by a sodium alginate particle-COL/hydroxyapatite composite scaffold, placental growth factor exhibited cartilage repair ability with a low-dose release [109]. However, due to the short half-lives, high cost, intolerance to sterilization conditions, sensitivity to pH, proneness to proteolysis, and rapid elimination in vivo [110], the long-term and stable release of growth factors relying on pore diffusion is hard to achieve by simply adding them into collagen-based biomaterials. Fixing the growth factors on the COL substrate guarantees their gradual release as COL is degraded. The strategy of fixing growth factors involves group complementarity, electrostatic interactions, affinity between substances and so on [45]. Moreover, the combination of BMSCs and insulin-like growth factor-1 or transforming growth factor β1 was shown to accelerate the healing of osteochondral defects [111]. However, the use of transforming growth factors can easily cause immune rejection, tumorigenesis, and heterogeneity. Autologous platelet-rich plasma is a useful substitute for promoting stem cell proliferation and cartilage formation through the TGF-B/SMAD signaling pathway. This plasma also inhibits the expression of the fibrocartilage biomarker COL I, and further advantages include its low

immunogenicity, easy availability and low cost [112]. In addition to growth factors, other compounds are capable of regulating cartilage repair. For example, hyaluronic acid was reported to stimulate MSCs to differentiate into cartilage and reduce the hypertrophy of chondrocytes during differentiation [113]. Matrine 3 showed similar performance [114].

Additionally, collagen can be used as a carrier scaffold for gene therapy. Tang et al. reported that COL I scaffolds effectively adsorbed plasmids containing Wnt5a genes. Wnt5a plasmids inhibited cartilage hypertrophy by affecting the expression of proteoglycan, COL II and SOX9 genes and then promoted osteochondral repair [76]. Qi et al. found that the implantation of a Wnt5a-COL composite scaffold significantly upregulated the PI3K pathway of BMSCs, activated the AKJ and JNK pathways, promoted the proliferation, migration and cartilage differentiation of BMSCs, and repaired defects in the area. A significant increase in the content of GAG and deposited COL indicated good interface integration and cartilage regeneration [81]. Hypoxia-inducible factor-1α promoted the differentiation into cartilage and matrix synthesis of stem cells in vitro and increased the survival rate of BMSCs under hypoxia and glucose deprivation. Treatment coupled with hypoxia-inducible factor-1α and COL scaffolds delayed abnormal bone hyperplasia and bone sclerosis of the subchondral bone [115].

4.2.2 Implanting cells

The combination of biomaterial scaffolds with key factors promotes host cells to regenerate cartilage in situ and is free from cell isolation and in vitro expansion. Implanting cells to increase the cell base of cartilage defects represents a promising strategy for cartilage repair, saving the additional time needed for the limited host cells to migrate and proliferate.

Before implantation, the cells need to be optimized according to in vitro and in vivo performance, including their types and resources. Chondrocytes, MSCs, and chondroprogenitors have been commonly used. There are limitations to using mature chondrocytes, such as the poor yield of chondrocytes isolated from autologous tissues, the necessity of being expanded and cultured in vitro before implantation, and the lack of inhibition to prevent chondrocytes from differentiating into fibroblasts [116]. The main challenges for in vitro culturing lie in maintaining the differentiating state of cells and ensuring the regeneration ability of damaged cartilage [91]. Excitingly, it was found that the protection of three-dimensional culture and specific cytokines help to maintain the cell phenotype of chondrocytes, which facilitates the generation of cartilage matrix containing autologous chondrocytes [117]. MSCs isolated from bone marrow or fat show good differentiation ability and are commonly applied to repair osteochondral injury; they are confirmed as ideal cell resources for cartilage regeneration owing to their easy acquisition from adult tissues and frequent expansion in vitro [118]. Nevertheless, depending on the age of the donor and the passage number, MSCs exhibit differentiation instability in vivo, and endochondral ossification may also occur [7, 119]. Progenitor cells from articular cartilage have been proven to be a potential substitute for BMSCs. Bauza et al. found that chondroprogenitors isolated from articular cartilage of patients with osteoarthritis expressed biomarkers of stem cells when cultured in vitro and demonstrated the differentiation ability of forming cartilage and osteogenesis [120].

The functional outputs of chondrocytes or MSCs from different sources are influenced by factors such as the age of the donor, heredity, and the microenvironment of the tissue. MSCs are widely derived from fat, bone marrow, synovium or skin. BMSCs have been used to manufacture engineered products for cartilage repair [121]. Donahue et al. studied the differences in function and components of new cartilage regenerated by rib chondrocytes in terms of age variations for the first time. The results showed that chondrocytes from young donors aged 0–25 could effectively undergo further passage and regenerate, self-assembling into new cartilage tissue with strong mechanical strength [122]. Based on a systematic comparison, the expression of biomarkers related to cell hypertrophy and cartilage in vivo was evaluated after implanting a COL scaffold load with BMSCs and umbilical cord blood-derived stem cells; BMSCs exhibited better chondrogenic potential [123]. Interestingly, autologous chondrocytes from the affected part of osteoarthritis were proven to have the potential to treat osteoarthritis assisted by COL scaffolds [124]. During the treatment of articular cartilage defects, Scioli et al. found that adipose-derived stem cells expressing CD146 showed spontaneous chondrogenic differentiation ability when cultured with a three-dimensional COL scaffold [125]. According to Cai et al., BMSCs are induced to differentiate into chondrocytes when treating articular cartilage defects with a suspension containing COL I and BMSCs. Hyaline-like cartilage was established after one month, and the cartilage surface was lubricated. After three months, the hypertrophic chondrocytes at the bottom of the cartilage promoted the regeneration of calcified cartilage. After six months, the continuous tide lines and complete calcification interface were recovered. Newly regenerated hyaline cartilage has similar thickness, matrix secretion, and collagen type and arrangement to that of adjacently native cartilage [126].

In addition to the above factors, the influence of scaffold structure on cell morphology and function should

be considered when using cell-collagen scaffold therapy. Yang et al. proposed that fibrous scaffolds have better performance in promoting the differentiation of stem cells into chondrocytes and alleviating calcification of ECM than porous scaffolds. Although fibrous scaffolds show strong protein adsorption and mass transfer capabilities, they cannot prevent the hypertrophy of chondrocytes induced by stem cells [127].

4.2.3 Tissue engineering technology

Tissue engineering technology includes scaffold preparation, cell breeding, incorporation with bioactive factors, etc. This approach provides a comprehensive repair strategy that combines both strategies above. According to the existing research, the tissue engineering strategy shows the most prominent effect on cartilage repair since normal surgical approaches can not provide long-term solution. The combination of seed cells and growth factors with scaffolds serves as the supplementary extracellular matrix with directed regeneration and differentiation potential [37]. However, the challenges that tissue engineering faces include all the problems existing in bioactive factor-COL scaffolds and cell-COL scaffold strategies; for example, the balance between the degradation rate of scaffolds and tissue growth, the influence of the degradation rate and products on tissue regeneration in vivo and in vitro, the changes in chondrocytes and MSCs during the process of dedifferentiation and expansion, the differences between newly generated tissues and native tissues, and so on [47]. In addition, clinical transformation requires careful selection of the most suitable biomaterials and technologies. Evaluations of the mechanical properties, biocompatibility, degradability, and regeneration potential of the scaffold are extremely necessary.

5 Comprehensive evaluations of cartilage repair by collagen-based biomaterials

Comprehensive evaluations of biomaterials are conducted by in vitro, in vivo and clinical trials. Except for the physicochemical assessments, in vitro evaluation focuses on cells, generally taking chondrocytes or MSCs as objects. In vivo tests rely on animal models. For small animals, non-load-bearing areas can be used to estimate the degradation, biocompatibility and interaction between the implants and the host tissue. Large animals are closer to clinical applications, giving more representative results by evaluating their weight-bearing areas with respect to the degradability and healing capacity of implanted biomaterials [21, 128]. Clinical trials are for larger animals and patients. We list the characterization indicators involved in previous studies, where the indicators reflect the specific properties and significance of the biomaterials.

5.1 Physicochemical properties

Focusing on collagen-based biomaterials for repairing cartilage defects, the physicochemical properties rely mainly on indicators such as the microstructure, swelling behavior, mechanical properties, and biodegradability.

First, to meet the high diffusion ability of living cells, growth factors, nutrients and metabolites in the scaffold, it is necessary to adjust the pores and density of collagen-based biomaterials [45]. As reported by Kohane et al., scaffolds with a total porosity of more than 90% are suitable for osteoblast growth, attachment and differentiation [129]. The interconnection between the pores is also important and affects the mechanical strength of the scaffold, cell survival, proliferation and migration, and the secretion of ECM [16]. Second, the swelling rate is another crucial indicator of the scaffold, which is closely related to the diffusion of signal molecules and nutrients. The swelling rate of the COL substrate is affected by the cross-linking degree [73, 130].

Another critical indicator is the mechanical properties. The COL substrate can simulate the mechanical properties of native cartilage at a macro level, which is essential for implanted COL scaffolds to integrate with cartilage defects to protect cells from compression or tensile stress caused by exercise. Under physiological load, the cartilage surface suffers from the highest liquid flow and the highest resistance to shear stress, the strain of which can reach 50%, while the strain in the deep zone is lower, accounting for only 0–5% without liquid flow [7]. However, the compression modulus in the deep zone is much higher. Taking the articular cartilage of cattle as an example, the compression modulus increases by 27 times from the joint surface to the deep region [131]. According to reports, the compressive modulus and tensile modulus of natural articular cartilage range from 0.1–2 MPa and 5–25 MPa, respectively [132, 133]. The mechanical properties of the collagen substrate are mainly measured by a universal tensile machine [73].

Biodegradability is a key criterion for the application of collagen-based biomaterials in vivo. The ideal collagen scaffold is directly degraded by enzymes and removed from the host tissues by kidney filtration or cell metabolism, with no need for surgical removal [134]. The degradation rate of the scaffold must be adjusted according to the rate of tissue formation. When the scaffold material degrades, new tissue is formed and takes over the mechanical load [45]. Owing to its natural biodegradability and nontoxicity, COL serves as an important raw material for cartilage repair. In in vitro tests, the

degradation examination of the COL scaffold can last for several months [19]. For in vivo experiments, the time for judging the regeneration and repair of cartilage is mostly calculated in months, and the repair effect of animal experiments is tested within three months to six months. The follow-up time of clinical trials is calculated in years, ranging from one to ten years. Therefore, the cross-linking parameters of COL should be adjusted to a specific degradation rate and time according to different implant sites while designing collagen-based biomaterials for cartilage repair.

5.2 Bioactivities for cartilage repair

Evaluating the ability of COL substrates in cartilage repair involves the following main aspects. The first aspect is the biological toxicity of the biomaterial itself, which directly indicates the effect of the biomaterial on cell viability and proliferation. The second aspect is the inductive ability when co-cultured with chondrocytes/stem cells, such as cell differentiation, ECM secretion and cartilage formation. The third aspect lies in the comprehensive performance of the biomaterial implanted in the cartilage defect in vivo, which is described by the morphology change of cell and tissue, the balance and integration between material degradation and cartilage regeneration, the difference between regenerated cartilage tissue and natural tissue, the adaptability to the complex metabolic environment and the investigation of abnormal events, etc. Herein, we sort out the critical methods, indication objects and the significance involved in the evaluation of the bioactivity of collagen-based biomaterials for cartilage repair from the perspectives of in vitro, in vivo and clinical tests (Table 1).

The morphology, viability and proliferation of cells cocultured with biomaterials can be determined by staining. The morphology and survival of cells on the biomaterials can be observed by laser confocal microscopy after calcein/pyridine iodide (FDA/PI) fluorescent staining. The cytotoxicity of biomaterials is quantified by MTT and CCK-8 tests, which characterize the proliferation of cells in a certain cycle [6, 14, 15]. Histological staining (Fig. 5e), immunohistochemical staining, quantification of the characteristic components and gene expression are generally used to evaluate the repair capacity of biomaterials in vitro and in vivo. Safranin-O/Fast Green staining identifies the total GAG content and the secretion of ECM. Hematoxylin and eosin (HE) staining is used for general observation of cell morphology and infiltration, regenerated tissue morphology, scaffold degradation, subchondral bone evaluation (bone morphology, bone filling and adhesion) and cartilage evaluation (joint surface, chondrocytes and glycosaminoglycans), as well as the type of regenerated cartilage. The thickness and content of newly formed cartilage can be quantitatively compared via software calculation [20, 58, 105]. Toluidine blue is a commonly used staining agent for chondrocytes and mastocytes in the clinic, which is helpful for the identification of hyaline cartilage by staining sulfate glycosaminoglycans (Fig. 4b) [83]. The type and fiber arrangement of COL can be recorded by polarized light microscopy [126]. In immunohistochemical analysis, the synthesis and secretion of COL I and COL II are reflected in shade staining and help to identify the type of regenerated cartilage [6, 58]. Alizarin red is used to assess calcium deposition in regenerating tissues [88]. Gross observation helps to identify the thickness, tide mark and subchondral bone of regenerated cartilage, as well as the integration ability of the implant biomaterial with the damaged tissue (Fig. 5(a)) [135]. In addition, the O'Driscoll score classifies the cartilage repair performance presented by the staining results [74].

Moreover, quantitative analysis of the characteristic components produced during cartilage formation is helpful in evaluating the cartilage repair effect of biomaterials. For example, the proliferation ability of the material on host cells and implanted cells can be assessed by the total DNA content [113]. Alkaline phosphatase activity (ALP) indicates the osteogenic differentiation ability of MSCs, which is stained by p-nitrophenyl phosphate [88]. The content of GAG can be quantitatively evaluated by dimethyl methylene blue (DMMB) (Fig. 5b) [59]. Considering the risk of an immune response caused by allogeneic collagen, the concentration of α-Gal heterologous antigen in the biomaterial can be determined, and decellularization treatment can reduce the α-Gal antigen content in the biomaterial [136].

The expression of cartilage repair-related genes is also an important indicator. Real-time reverse transcription polymerase chain reaction is the main detection method [75]. The following genes have been classified: (1) Chondrogenic genes: NCDH, TGFB1, SOX9, ACAN, COL2A1, GAPDH, COMP, BMP-2, MMP-13, VCAN, ADAMTS-5, TIMP4, FGF2, FGFR1 and FGFR3 [6, 74, 76, 90, 108, 124]; (2) Osteogenesis-specific genes: COL1A1, OCN/BGLAP, OSN/SPP1, COL3A1 and RUNX2 [59, 78, 106]; (3) Hypertrophy biomarker: COL10A1 [127, 137]; (4) Apoptosis and proliferation regulators: BCL2, CASP3, MCM5 and CCND1 [78]; and (5) Abnormal events: MMP-13, ADAMTS-5, FGFR1 and TIMP4 [30, 108, 124]. Moreover, high-throughput sequencing transcription profiles can comprehensively evaluate the impact of implanted materials on the upregulation and downregulation of genes in surrounding tissues [86].

In animal models, evaluations of the repair capacity and regeneration ability of the scaffold are performed

Table 1 General methods used to evaluate the bioactivities of biomaterials

Characterizations	Reagents or objects	Potential indications	References
Cell staining	FDA-PI	Morphology, viability and proliferation of cells. Cytocompatibility of biomaterials	[6]
	MTT, CCK8		[15, 18]
Histochemical staining	Safranin O-Fast Green	Relative content of GAG. Morphology of chondrocytes. Cellular infiltration in defect sites. Regeneration of hyaline cartilage	[6, 16, 58]
	Toluidine Blue	Secretion of GAG. Regeneration of hyaline cartilage	[105]
	Hematoxylin and Eosin	Morphology of cell nucleus, ECM and tissue. Identification of regeneration of tissue, degraded scaffold, and type of regenerated cartilage. Evaluation of cartilage and subchondral bone	
	Picrosirius Red	Identification of collagen type and the arrangement of collagen fibrils	[124]
	Alizarin Red S	Evaluation of calcium deposit and calcified cartilage	[88]
Immunohistochemical staining	COL II, COL I	Judgement of regenerated cartilage according to their expression	[58]
Quantification	Total DNA	Proliferation of cells	[83, 113]
	Total GAG	Production of ECM. Reflection of tissue development	[59, 83]
	Total COL		
	COL II		
Enzyme activity	Alkaline phosphatase	Ability of cells on osteogenic differentiation	[59]
Gene expression	*Sox9*, NCDH, ACAN, *COL2A1*, BMP-2, COMP	Biomarkers of cartilage regeneration. Evaluation of cartilage forming capacity	[6, 75, 90, 124, 127]
	FGFR3	Regulation on the bone formation. Evaluation of cartilage forming capacity	[108]
	OPN (*SPP1*), *Osx*, OCN (*Bglap*), *Runx-2*	Specific genes of osteogenesis. Determination of stem cell differentiation and chondrocyte maturation	[59, 78, 90, 106]
	COL1A1,	Specific genes of osteogenesis. Biomarker of cartilage forming and fibrous cartilage	[6, 90, 136]
Gene expression	FGF2	Biomarker of subchondral bone. Progress of tissue development	[108]
	MMP-13, ADAMTS-5	Degradation of COL II and ECM. Flection of cartilage replacement	[30, 33, 124]
	COL10A1	Biomarker of cell hypertrophy	[125]
	TIMP4	Indicator of inflammation	[108]
	FGFR1	Indicator of tumorigenesis	
	GAPDH	Specific gene indicating cell viability	[6, 124]
	Bcl2, *Casp3*	Specific gene indicating cell apoptosis	[78]
	MCM5, *CCND1*	Specific gene indicating cell proliferation	
Antigen detection	α-Gal	Reflection of immunogenicity	[136]

through macroscopic, microtomography, histomorphometry and immunohistochemical analyses. In addition to the abovementioned methods, several methods such as macroscopic evaluation, naked eye observation combined with the ICRS score, and micro-CT are suitable to evaluate the flatness and closure of the cartilage surface, inflammation, degradation of the scaffold, new bone formation, adverse tissue degeneration and other circumstances (Fig. 5d) [46, 58, 137]. Nanoindentation instruments provide a novel measurement for the biomechanical properties of regenerated cartilage, combining fiber-optical Fabry–Perot interferometry with a monolithic cantilever-based probe. The local microelasticity of low-modulus materials can be examined with high accuracy and precision [113]. Saukko et al. proposed an advanced nondestructive quantitative technology, namely, quantitative dual-energy computed tomography, which can assess the changes around trauma sites and quantify proteoglycans, thereby assisting in judging the degree and severity of cartilage damage. Although the authors only focused on the assessment of damaged cartilage, this approach can also monitor the repair process of cartilage [138].

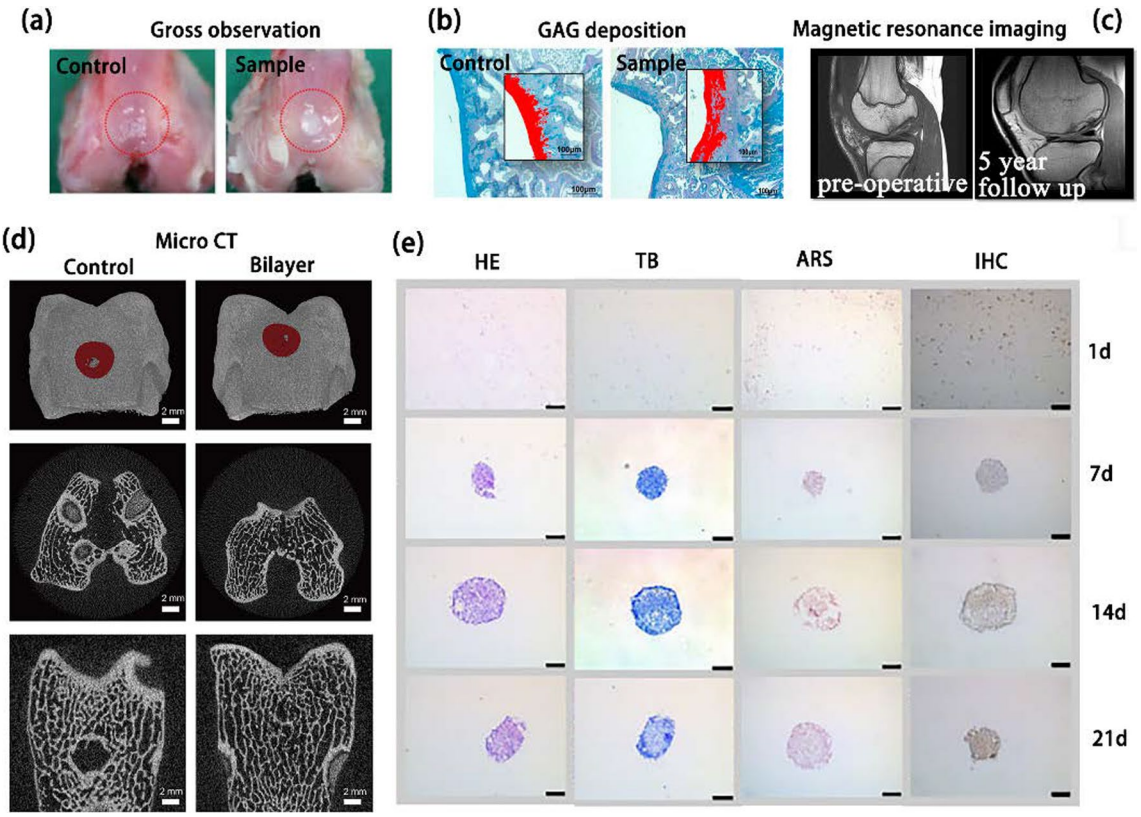


Fig. 5 General characterization for cartilage repairs with respect to direct observing and bio-indicators recognizing. **a** Gross observation and records of defect repairing. (Adapted from ref. [135], copyright 2021, with permission from the Springer Nature.) **b** Histochemical evaluation of deposition of GAG by Alcian Blue staining. (Adapted from ref. [93], copyright 2020, with permission from MDPI.) **c** Magnetic resonance imaging of comparison of an osteochondral graft at ten-year follow-up investigation. The alterations of subchondral bone and graft filling reflected the repairing performance by graft. (Adapted from ref. [23], copyright 2021, with permission from Springer Open.) **d** Representative Micro-computed tomography (Micro-CT) images defect site and surrounding area. Bilayer represents the scaffold fabricated by human-like-collagen, hyaluronan and nano-hydroxyapatite particles. (Adapted from ref. [59], copyright 2020, with permission from Science China Press and Springer Nature) **e** Major staining methods for bio-indicator evaluations. HE used Hematoxylin–eosin staining (HE) describes the general morphology of cell and regenerated tissue. Toluidine blue (TB) identifies the hyaline cartilage by staining sulfate glycosaminoglycans. Alizarin red staining (ARS) assesses the calcium deposition. Immunohistochemical staining supplies the synthesis and secretion of collagen type I and II. (Adapted from ref. [103], copyright 2020, with permission from Oxford)

5.3 Clinical diagnosis

In clinical trials, the evaluation of the repair capacity of biomaterials for cartilage defects is mainly based on MRI images and the histological score of biopsy in follow-up investigations (Fig. 5c). With the help of appropriate scoring methods and standards, the degree of repair is scored and graded. Therefore, judgment on the effectiveness and durability of cartilage repair strategies should be given from a clinical perspective. The clinical scoring standards that can be referred to include VAS, IKDC, Lysholm, Tegner, KOOS, O'Driscoll, MODS, ICRS I and ICRS II, MOCART, etc. [104, 118, 139–141].

In particular, the grading object of MOCART is MRI [142], and the scoring angles are as follows: degree of defect repair and filling, integration with neighboring zones, repair tissue surface quality, repair tissue structure, repair tissue signal intensity, subchondral lamina, subchondral bone, adhesions, and synovitis [104, 140]. ICRS II is composed of 14 criteria for evaluating parameters related to chondrocyte phenotype and tissue structure. Mainil-Varlet et al. compared ICRS II with ICRS I and MODS and showed that ICRS II has improved readability over the current histological cartilage repair scoring system [139]. ICRS I scores showed poor reliability in animal cartilage repair models [143]. KOOS and IKDC are both scoring systems for knee joint injury treatment [140]. The IKDC has two types of scoring systems: subjective and objective. The Tegner-Lysholm knee joint score scale and the Tegner activity level scale have been used to assist the IKDC analysis [23, 24]. In addition to

MRI, computed tomography scans are used in clinical diagnosis. The criteria are flexible to some extent, such as the percentage of filling of the implant, the homogeneity of the newly formed cartilage, the ability to integrate with tissue, and the number of tissues surrounding the implants. Finally, the overall score is calculated in a blind manner [144]. Biomechanical testing of regenerated cartilage can be performed simultaneously during arthroscopy. Peterson et al. employed an electromechanical tracking probe to measure the hardness of cartilage [145].

6 Conclusions and prospects

Therapy of cartilage defects is a chronic and difficult clinical challenge. The crux of the challenge lies in the highly complex structure and functional differences of cartilage. With further research on cartilage damage and continuous exploration of strategies for defect repair, the preparation and functionalization of collagen-based biomaterials for cartilage repair has a certain research foundation and design framework, which fully exploits the excellent biocompatibility and biological characteristics of collagen and simultaneously strengthens the physicochemical properties of collagen substrates. Moreover, supplementation with bioactive substances promotes the targeted secretion of ECM from host cells or exogenous cells, thereby accelerating cartilage regeneration and tissue integration. Collagen-based biomaterials developed in three-dimensional and porous structure, such as hydrogels, are proven to be promising and prospective in cartilage repair.

After overviewing the design strategies and repair capabilities of existing collagen-based biomaterials for cartilage repair, the advantages of biomimetic multilayer three-dimensional scaffolds are affirmed regardless of whether they are cell-free COL scaffolds, cell-COL scaffolds or tissue engineering scaffolds. Nevertheless, there are still many problems to be solved and explored: (1) matching the dependence on the scaffold during cartilage regeneration with the degradation rate and biomechanical strength of the scaffold; (2) describing the influence of complex components in biomaterials on the circulatory system during and after degradation in the body; (3) stabilizing the load and maintaining the integration of bioactive substances; (4) creating dedifferentiation technology to limit cell expansion and culture in vitro; (5) developing a directional induction method for the regenerating cartilage type; (6) verifying the difference between animal models and actual conditions in the human body; and (7) exploring good solvents for collagen.

Abbreviations

ACAN: Aggrecan; ACI: Autologous/allogeneic chondrocyte implantation; ADAMTS-5: A disintegrin and metalloproteinase with thrombospondin 5; BDDGE: 1,4-Butanediol diglycidyl ether; BMP-2: Bone morphogenetic protein-2; BMS: Bone marrow stimulation; BMSCs: Bone marrow mesenchymal stem cells; CCK8: Cell counting kit-8; COL: Collagen; COLI: Collagen type I; COLII: Collagen type II; COMP: Cartilage oligomeric matrix protein; ECM: Extracellular matrix; EDC: N-(3-dimethylaminopropy)-N′-ethylcarbodimmide; FDA: Fluorescein diacetate; FGF2: Fibroblast growth factor 2; FGFR1: Fibroblast growth factor receptor 1; FGFR3: Fibroblast growth factor receptor 3; GAG : Glycosaminoglycan; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; HE: Hematoxylin–eosin; ICRS: International Cartilage Repair Society; MACI: Matrix-induced autologous chondrocyte implantation; MMP-13: Matrix metalloproteinase-13; MRI: Magnetic resonance imaging; MSCs: Mesenchymal stem cells; MTT: Methyl thiazolyl tetrazolium; NCDH: N-cadherin; NHS: N-Hydroxysuccinimide; OCN: Osteocalcin; OPN: Osteopontin; PCL: Polycaprolactone; PEDOT: 3,4-Ethylenediyangthiophene; PI: Propidium iodide; PLA: Polylactic acid; QDs: Quantum dots; ROS: Reactive oxygen species; TIMP4: Tissue inhibitor of metalloproteinases 4.

Acknowledgements

We gratefully acknowledge financial support of this work by the Fundamental Research Funds from the Central University [2662018JC019], the Huazhong Agricultural University-Agricultural Genomics Institute at Shenzhen Cooperation Fund (SZYJY2021009) and the China Agriculture Research System (No. CARS-45-28).

Authors' contributions

XY was a major contributor in writing the manuscript and collecting all relevant literatures. HZ was a major contributor in drawing all figures and tables. YM, SX and YH contributed to designing the framework of this review and revising the manuscript. All authors read and approved the final manuscript.

Funding

We gratefully acknowledge financial support of this work by Fundamental Research Funds from the Central University (2662018JC019), Huazhong Agricultural University-Agricultural Genomics Institute at Shenzhen Cooperation Fund (SZYJY2021009) and China Agriculture Research System (No. CARS-45-28).

Availability of data and materials

Not applicable.

Declarations

Competing interests

The authors declare that they have no competing interests.

Author details

[1]College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, People's Republic of China. [2]Shenzhen Institute of Nutrition and Health, Huazhong Agricultural University, Wuhan 430070, Hubei, People's Republic of China. [3]Bioactive Peptide Technology Hubei Engineering Research Center, No.195 Dongfang Ave, Jingzhou 434000, Hubei, People's Republic of China.

Received: 14 December 2021 Accepted: 22 February 2022
Published online: 28 March 2022

References

1. Wang Y, Wei L, Zeng L, He D, Wei X. Nutrition and degeneration of articular cartilage. Knee Surg Sports Tr A. 2013;21(8):1751–62.
2. Smith B, Sigal IR, Grande DA. Immunology and cartilage regeneration. Immunol Res. 2015;63(1–3):181–6.
3. Turnbull G, Clarke J, Picard F, Zhang W, Shu W. 3D biofabrication for soft tissue and cartilage engineering. Med Eng Phys. 2020;82:13–39.

4. Mollon BR, Kandel JC, Theodoropoulos J. The clinical status of cartilage tissue regeneration in humans. Osteoarthr Cartilage. 2013;21(12):1824–33.
5. Koh JL, Kowalski A, Lautenschlager E. The effect of angled osteochondral grafting on contact pressure: a biomechanical study. Am J Sports Med. 2006;34(1):116–9.
6. Li X, Teng Y, Liu J, Hai L, Zhang X. Chondrogenic differentiation of BMSCs encapsulated in chondroinductive polysaccharide/collagen hybrid hydrogels. J Mater Chem B. 2017;5(26):5109–19.
7. Armiento AR, Stoddart MJ, Alini M, Eglin D. Biomaterials for articular cartilage tissue engineering: learning from biology. Acta Biomater. 2018;65:1–20.
8. Cucchiarini M, Madry H. Biomaterial-guided delivery of gene vectors for targeted articular cartilage repair. Nat Rev Rheumatol. 2019;15(1):18–29.
9. Wu Z, Korntner SH, Mullen AM, Zeugolis DI. In the quest of the optimal chondrichthyan for the development of collagen sponges for articular cartilage. J Sci-Adv Mater Dev. 2021;6:390–8.
10. Chen HH, Yang XH, Liao YY, Zeng XW, Liang PH, Kang N, Tan JR, Liang ZP. MRI and histologic analysis of collagen type II sponge on repairing the cartilage defects of rabbit knee joints. J Biomed Mater Res B Appl Biomater. 2011;96(2):267–75.
11. Wess TJ, Parry DAD, Squire JM. Collagen fibril form and function. Adv Protein Chem. 2005;70:341.
12. Pustlauk W, Paul B, Gelinsky M, Bernhardt A. Jellyfish collagen and alginate: combined marine materials for superior chondrogenesis of hMSC. Mat Sci Eng C. 2016;64:190–8.
13. Wang J, Ying Y, Sun X, Liu D, Huang C. Biomimetic cartilage scaffold with orientated porous structure of two factors for cartilage repair of knee osteoarthritis. Artif Cells Nanomed Biotechnol. 2019;47(1):1710–21.
14. Chevallay B, Herbage D. Collagen-based biomaterials as 3D scaffold for cell cultures: applications for tissue engineering and gene therapy. Med Biol Eng Comput. 2000;38:211–8.
15. Ricollanos GA, Borregogonz S, Moncayodonoso M, Becerra J, Visser R. Collagen type I biomaterials as scaffolds for bone tissue engineering. Polymers. 2021;13:599.
16. Elkhoury K, Morsink M, Sanchez-Gonzalez L, Kahn C, Arab-Tehrany E. Biofabrication of natural hydrogels for cardiac, neural, and bone tissue engineering applications. Bioact Mater. 2021;6(11):3904–23.
17. Thunsiri K, Pitjamit S, Pothacharoen P, Pruksakorn D, Nakkiew W, Wattanutchariya W. The 3D-printed bilayer's bioactive-biomaterials scaffold for full-thickness articular cartilage defects treatment. Materials. 2020;13:3417.
18. Hashemi S, Mohammadi AL, Farzad-Mohajeri S, Rezai Rad M, Fahimipour F, Ardeshirylajimi A, Dashtimoghadam E, Salehi M, Soleimani M, Mehdi Dehghan M, Tayebi L, Khojasteh A. Comparison of osteogenic differentiation potential of induced pluripotent stem cells and buccal fat pad stem cells on 3D-printed HA/beta-TCP collagen-coated scaffolds. Cell Tissue Res. 2021;384(2):403–21.
19. Lee CF, Hsu YH, Lin YC, Nguyen TT, Chen HW, Nabilla SC, Hou SY, Chang FC, Chung RJ. 3D Printing of collagen/oligomeric proanthocyanidin/oxidized hyaluronic acid composite scaffolds for articular cartilage repair. Polymers. 2021;13:3123.
20. Bauza-Mayol G, Quintela M, Brozovich A, Hopson M, Shaikh S, Cabrera F, Shi A, Niclot FB, Paradiso F, Combellack E, Jovic T, Rees P, Tasciotti E, Francis LW, Mcculloch P, Taraballi F. Biomimetic scaffolds modulate the posttraumatic inflammatory response in articular cartilage contributing to enhanced neoformation of cartilaginous tissue *in vivo*. Adv Healthc Mater. 2021; e2101127.
21. Meng X, Ziadlou R. Grad S, Alini M, Wang X. Animal models of osteochondral defect for testing biomaterials. Biochem Res Int. 2020; e9659412.
22. Uday Chadrika K, Kacha S, Nair AS, Jamwal VS, Sandilya S, Singh S. Tissue engineering of cartilage using collagen scaffold enriched with plant polysaccharides. Cartilage. 2021; e19476035211007899.
23. Ricci M, Tradati D, Maione A, Uboldi FM, Usellini E, Berruto M. Cell-free osteochondral scaffolds provide a substantial clinical benefit in the treatment of osteochondral defects at a minimum follow-up of 5 years. J Exp Orthop. 2021; 8(62).
24. Di Martino A, Perdisa F, Filardo G, Busacca M, Kon E, Marcacci M, Zaffagnini S. Cell-Free Biomimetic osteochondral scaffold for the treatment of knee lesions: clinical and imaging results at 10-year follow-up. Am J Sports Med. 2021;49(10):2645–50.
25. Liu XH, Zheng C, Luo XM, Wang XC, Jiang HE. Recent advances of collagen-based biomaterials: multi-hierarchical structure, modification and biomedical applications. Mat Sci Eng C-Mater. 2019;99:1509–22.
26. Kluyskens L, Debieux P, Wong KL, Krych AJ, Saris DBF. Biomaterials for meniscus and cartilage in knee surgery: State of the a. rt. J ISAKOS. 2021 (available online).
27. Lin ZD, Tao Y, Huang Y, Xu T, Niu W. Applications of marine collagens in bone tissue engineering. Biomed Mater. 2021; 16(4):042007.
28. Zhao W, Jin X, Cong Y, Liu YY, Fu J. Degradable natural polymer hydrogels for articular cartilage tissue engineering. J Chem Technol Biot. 2013;88(3):327–39.
29. Robin Poole A, Kojima T, Yasuda T, Mwale F, Kobayashi M, Laverty S. Composition and structure of articular cartilage: a template for tissue repair. Clin Orthop Relat R. 2001;391S:S26–33.
30. Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. Sports Health. 2009;1(6):461–8.
31. Torzilli PA, Grigiene R, Borrelli J Jr, Helfet DL. Effect of impact load on articualr cartilage: cell metabolism and viability, and matrix water content. J Biomech Eng. 1999;232:433–41.
32. Gordey EE, Wong IH. Cartilage repair in the hip. Ann Joint. 2018;3:24–24.
33. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum. 2012;64(6):1697–707.
34. He L, Chen Y, Ke Z, Pang M, Yang B, Feng F, Wu Z, Liu C, Liu B, Zheng X, Wu T, Shu T. Exosomes derived from miRNA-210 overexpressing bone marrow mesenchymal stem cells protect lipopolysaccharide induced chondrocytes injury via the NF-kappaB pathway. Gene. 2020;751:144764.
35. Becerra J, Andrades JA, Guerado E, Zamora-Navas P, López-Puertas JM, Reddi AH. Articular cartilage: structure and regeneration. Tissue Eng Part B Rev. 2010;16(6):617–27.
36. Gobbi A, Scotti C, Lane JG, Peretti GM. Fresh osteochondral allografts in the knee: only a salvage procedure? Ann Transl Med. 2015;3(12):164.
37. Kwon H, Brown WE, Lee CA, Wang D, Paschos N, Hu JC, Athanasiou KA. Surgical and tissue engineering strategies for articular cartilage and meniscus repair. Nat Rev Rheumatol. 2019;15(9):550–70.
38. Berkowitz JJ, Ferkel RD. New and emerging techniques in cartilage repair: matrix-induced autologous chondrocyte implantation (MACI). Sports Injuries Foot Ankle. 2019; 125–131.
39. Roberts S, Menage J, Sandell LJ, Evans EH, Richardson JB. Immunohistochemical study of collagen types I and II and procollagen IIA in human cartilage repair tissue following autologous chondrocyte implantation. Knee. 2009;16(5):398–404.
40. Kwan H, Chisari E, Khan WS. Cell-free scaffolds as a monotherapy for focal chondral knee defects. Materials. 2020;13(2):306.
41. Sun H, Hu S, Zhang Z, Lun J, Liao W, Zhang Z. Expression of exosomal microRNAs during chondrogenic differentiation of human bone mesenchymal stem cells. J Cell Biochem. 2019;120(1):171–81.
42. Shimomura K, Rothrauff BB, Tuan RS. Region-specific effect of the decellularized meniscus extracellular matrix on mesenchymal stem cell-based meniscus tissue engineering. Am J Sports Med. 2017;45(3):604–11.
43. Chu W, Hu G, Peng L, Zhang W, Ma Z. The use of a novel deer antler decellularized cartilage-derived matrix scaffold for repair of osteochondral defects. J Biol Eng. 2021;15:23.
44. Lim T, Tang Q, Zhu ZZ, Feng Y, Zhan S, Wei XJ, Zhang CQ. A decellularized scaffold derived from squid cranial cartilage for use in cartilage tissue engineering. J Mater Chem B. 2020;8(20):4516–26.
45. Censi R, Dubbini A, Matricardi P. Bioactive hydrogel scaffolds—advances in cartilage regeneration through controlled drug delivery. Curr Pharm Des. 2015;21:1545–55.
46. Crovace AM, Giancamillo AD, Gervaso F, Mangiavini L, Zani D, Scalera F, Palazzo B, Izzo D, Agnoletto M, Domenicucci M, Sosio C, Sannino A, Giancamillo MD, Peretti GM. Evaluation of *in vivo* response of three biphasic scaffolds for osteochondral tissue regeneration in a sheep model. Vet Sci. 2019;6:90.
47. Maia FR, Carvalho MR, Oliveira JM, Rui LR. Tissue engineering strategies for osteochondral repair. Adv Exp Med Biol. 2018;1059:353–71.
48. Rowland CR, Glass KA, Ettyreddy AR, Gloss CC, Matthews JRL, Huynh NPT, Guilak F. Regulation of decellularized tissue remodeling via

scaffold-mediated lentiviral delivery in anatomically-shaped osteochondral constructs. Biomaterials. 2018;177:161–75.
49. Herrera Millar VR, Mangiavini L, Polito U, Canciani B, Nguyen VT, Cirillo F, Anastasia L, Peretti GM, Modina SC, Di Giancamillo A. Hypoxia as a stimulus for the maturation of meniscal cells: highway to novel tissue engineering strategies? Int J Mol Sci. 2021;22:6905.
50. Jahanbakhsh A, Nourbakhsh MS, Bonakdar S, Shokrgozar MA, Haghighipour N. Evaluation of alginate modification effect on cell-matrix interaction, mechanotransduction and chondrogenesis of encapsulated MSCs. Cell Tissue Res. 2020;381(2):255–72.
51. Dos Santos DA, de Guzzi Plepis AM, da Conceição Amaro Martins V, Cardoso GBC, Santos AR Jr, Iatecola A, Andrade TN, Monteiro FM, Calegari ARA, Chacon EL, Cunha MR. Effects of the combination of low-level laser therapy and anionic polymer membranes on bone repair. Lasers Med Sci. 2020;35(4):813–21.
52. Polito U, Peretti GM, Di Giancamillo M, Boschetti F, Carnevale L, Veronesi MC, Sconfienza LM, Agnoletto M, Mangiavini L, Modina SC, Di Giancamillo A. Meniscus matrix remodeling in response to compressive forces in dogs. Cells. 2020;9:265.
53. Buehler MJ. Nature designs tough collagen: explaining the nanostructure of collagen fibrils. PANS. 2006;103(33):12285–90.
54. Zhao CX, Xiao YL, Ling SJ, Pei Y, Ren B. Structure of Collagen. Methods Mol Biol. 2021;2347:17–25.
55. Shoulders MD, Raines RT. Collagen structure and stability. Annu Rev Biochem. 2009;78:929–58.
56. Sobczak-Kupiec A, Drabczyk A, Florkiewicz W, Głąb M, Kudłacik-Kramarczyk S, Słota D, Tomala A, Tyliszczak B. Review of the applications of biomedical compositions containing hydroxyapatite and collagen modified by bioactive components. Materials. 2021;14:2096.
57. Salvatore L, Gallo N, Natali ML, Terzi A, Sannino A, Madaghiele M. Mimicking the hierarchical organization of natural collagen: toward the development of ideal scaffolding material for tissue regeneration. Front Bioeng Biotech. 2021;9:644595.
58. Salvatore L, Gallo N, Natali ML, Terzi A, Sannino A, Madaghiele M. mi the development of ideal scaffolding material for tissue regeneration. Front Bioeng Biotech. 2021;9:644595.
59. Liu KQ, Liu YN, Duan ZG, Ma XX, Fan DD. Sci China Technol Sci. 2020;64(4):793–805.
60. Zhou HC, Chen R, Wang JP, Lu J, Yu T, Wu XB, Xu SC, Li ZH, Jie C, Cao RF, Yang YF, Li YQ, Mneg DP. Biphasic fish collagen scaffold for osteochondral regeneration. Mat Design. 2020;195:108947.
61. Abou Neel EA, Bozec L, Knowles JC, Syed O, Mudera V, Day R, Hyun JK. Collagen-emerging collagen based therapies hit the patient. Adv Drug Deliv Rev. 2013;65(4):429–56.
62. Sorushanova A, Delgado LM, Wu ZN, Shologu N, Kshirsagar A, Raghunath R, Mullen AM, Bayon Y, Pandit A, Raghunath M, Zeugolis DI. 3 development. Adv Mater. 2019;31:e1801651.
63. Ehrmann RL, Gey GO. The growth of cells on a transparent gel of reconstituted rat-tail collagen. JNCI-J Natl Cancer I. 1956;16(6):1375–403.
64. Chen Y, Ma M, Cao H, Wang Y, Xu Y, Teng Y, Sun Y, Liang J, Fan Y, Zhang X. Identification of endogenous migratory MSC-like cells and their interaction with the implant materials guiding osteochondral defect repair. J Mater Chem B. 2019;7(25):3993–4007.
65. Walters BD, Stegemann JP. Strategies for directing the structure and function of three-dimensional collagen biomaterials across length scales. Acta Biomater. 2014;10(4):1488–501.
66. Taubenberger AV, Woodruff MA, Bai H, Muller DJ, Hutmacher DW. The effect of unlocking RGD-motifs in collagen I on pre-osteoblast adhesion and differentiation. Biomaterials. 2010;31(10):2827–35.
67. Mehta M, Madl CM, Lee S, Duda GN, Mooney DJ. The collagen I mimetic peptide DGEA enhances an osteogenic phenotype in mesenchymal stem cells when presented from cell-encapsulating hydrogels. J Biomed Mater Res A. 2015;103(11):3516–25.
68. Zhang D, Wu X, Chen J, Lin K. The development of collagen based composite scaffolds for bone regeneration. Bioact Mater. 2018;3(1):129–38.
69. Zelenski NA, Leddy HA, Sanchez-Adams J, Zhang J, Bonaldo P, Liedtke W, Guilak F. Type VI collagen regulates pericellular matrix properties, chondrocyte swelling, and mechanotransduction in mouse articular cartilage. Arthritis Rheumatol. 2015;67(5):1286–94.
70. Wotton SF, Duance VC. Type III collagen in normal human articular cartilage. Histochem J. 1994;26(5):412–6.
71. Wu JJ, Weis MA, Kim LS, Eyre DR. Type III collagen, a fibril network modifier in articular cartilage. J Biol Chem. 2010;285(24):18537–44.
72. Hosseininia S, Weis MA, Rai J, Kim L, Funk S, Dahlberg LE, Eyre DR. Evidence for enhanced collagen type III deposition focally in the territorial matrix of osteoarthritic hip articular cartilage. Osteoarthr Cartilage. 2016;24(6):1029–35.
73. Guo Y, Yuan T, Xiao ZW, Tang PP, Xiao YM, Fan YJ, Zhang XD. Hydrogels of collagen/chondroitin sulfate/hyaluronan interpenetrating polymer network for cartilage tissue engineering. J Mater Sci Mater Med. 2012;23(9):2267–79.
74. Kilmer CE, Battistoni CM, Cox A, Breur GJ, Panitch A, Liu JC. Collagen type I and II blend hydrogel with autologous mesenchymal stem cells as a scaffold for articular cartilage defect repair. ACS Biomater Sci Eng. 2020;6:3464–76.
75. Yang J, Tang Z, Liu Y, Luo Z, Xiao Y, Zhang X. Comparison of chondroinductivity between collagen and hyaluronic acid hydrogel based on chemical/physical microenvironment. Int J Biol Macromol. 2021;182:1941–52.
76. Tang RF, Zhou XZ, Niu L, Qi YY. Type I collagen scaffold with WNT5A plasmid for *in situ* cartilage tissue engineering. Biomed Mater Eng. 2021; 34366316.
77. Thavornyutikarn B, Chantarapanich N, Sitthiseripratip K, Thouas GA, Chen Q. Bone tissue engineering scaffolding: computer-aided scaffolding techniques. Prog Biomater. 2014;3:61–102.
78. Amann E, Amirall A, Franco AR, Poh PSP, Sola Dueñas FJ, Fuentes Estévez G, Leonor IB, Reis RL, van Griensven M, Balmayor ER. A graded, porous composite of natural biopolymers and octacalcium phosphate guides osteochondral differentiation of stem cells. Adv Healthc Mater. 2021; 10, e2001692.
79. Lan WW, Xu MJ, Qin M, Cheng YZ, Zhao Y, Huang D, Wei XC, Guo YC, Chen WY. Physicochemical properties and biocompatibility of the bi-layer polyvinyl alcohol-based hydrogel for osteochondral tissue engineering. Mater Design. 2021;204:109652.
80. Putri NRE, Wang XH, Chen Y, Li XM, Kawazoe N, Chen GP. Preparation of PLGA-collagen hybrid scaffolds with controlled pore structures for cartilage tissue engineering. Prog Nat Sci. 2020;30(5):642–50.
81. Qi YY, Zhang WK, Li GQ, Niu L, Zhang YX, Tang RF, Feng G. An oriented-collagen scaffold including *Wnt5a* promotes osteochondral regeneration and cartilage interface integration in a rabbit model. FASEB J. 2020;34(8):11115–32.
82. Feng X, Xu PF, Shen T, Zhang YH, Ye J, Gao CY. Influence of pore architectures of silk fibroin/collagen composite scaffolds on the regeneration of osteochondral defects *in vivo*. J Mater Chem B. 2020;8(3):391–405.
83. She YL, Fan ZW, Wang L, Li YZ, Sun WY, Tang H, Zhang L, Wu L, Zheng H, Chen C. 3D printed biomimetic pcl scaffold as framework interspersed with collagen for long segment tracheal replacement. Front Cell Dev Biol. 2021;9:629796.
84. Schwab A, Hélary C, Richards RG, Alini M, Eglin D, D'Este M. Tissue mimetic hyaluronan bioink containing collagen fibers with controlled orientation modulating cell migration and alignment. Mater Today Bio. 2020;7:100058.
85. Kim M, Choe YE, Kim GH. Injectable hierarchical micro/nanofibrous collagen-based scaffolds. Chem Eng J. 2019;365:220–30.
86. Ji TJ, Feng B, Shen J, Zhang M, Hu YQ, Jiang AX, Zhu DQ, Chen YW, Ji W, Zhang Z, Zhang H, Li F. An avascular niche created by axitinib-loaded pcl/collagen nanofibrous membrane stabilized subcutaneous chondrogenesis of mesenchymal stromal cells. Adv Sci. 2021;8:e2100351.
87. Yu XY, Tang CE, Xiong SB, Yuan QJ, Gu ZP, Li Z, Hu Y. Modification of collagen for biomedical applications: a review of physical and chemical methods. Curr Org Chem. 2016;20:1797–812.
88. Bernhardt A, Paul B, Gelinsky M. Biphasic scaffolds from marine collagens for regeneration of osteochondral defects. Mar Drugs. 2018;16:91.
89. Marin MM, Albu Kaya MG, Vlasceanu GM, Ghitman J, Radu IC, Iovu H. The effect of cross-linking agents on the properties of type II collagen biomaterials. Mater Plast. 2020;57(4):166–80.
90. Zhang XH, Zhou J, Ying HY, Zhou YT, Lai JH, Chen JH. Glycogen as a cross-linking agent of collagen and nanohydroxyapatite to form hydrogels for bmsc differentiation. ACS Sustain Chem Eng. 2020;8(4):2106–14.
91. Rigogliuso S, Salamone M, Barbarino E, Barbarino M, Nicosia A, Ghersi G. Production of injectable marine collagen-based hydrogel for the

maintenance of differentiated chondrocytes in tissue engineering applications. Int J Mol Sci. 2020;21:5798.
92. Santarella F, Simpson CR, Lemoine M, McGrath S, Cavanagh B, Smith A, Murphy CM, Garlick JA, O'Brien FJ, Kearney CJ. The lubricating effect of iPS-reprogrammed fibroblasts on collagen-GAG scaffolds for cartilage repair applications. J Mech Behav Biomed Mater. 2021;114:e104174.
93. Szychlinska MA, Calabrese G, Ravalli S, Dolcimascolo A, Castrogiovanni P, Fabbi C, Puglisi C, Lauretta G, Rosa MD, Castorina A, Parenti R, Musumeci G. Evaluation of a cell-free collagen type I-based scaffold for articular cartilage regeneration in an orthotopic rat model. Materials. 2020;13:2369.
94. Zheng L, Liu SG, Cheng XJ, Qin ZN, Lu ZH, Zhang K, Zhao JM. Intensified stiffness and photodynamic provocation in a collagen-based composite hydrogel drive chondrogenesis. Adv Sci. 2019;6:1900099.
95. Lu Z, Liu SJ, Le YG, Qin ZN, He MW, Xu FB, Zhu Y, Zhao JM, Mao CB, Zheng L. An injectable collagen-genipin-carbon dot hydrogel combined with photodynamic therapy to enhance chondrogenesis. Biomaterials. 2019;218:119190.
96. Sridharan R, Ryan EJ, Kearney CJ, Kelly DJ, O'Brien FJ. Macrophage polarization in response to collagen scaffold stiffness is dependent on cross-linking agent used to modulate the stiffness. ACS Biomater Sci Eng. 2019;5(2):544–52.
97. Roffi A, Kon E, Perdisa F, Fini M, Di Martino A, Parrilli A, Salamanna F, Sandri M, Sartori M, Sprio S, Tampieri A, Marcacci M, Filardo G. A composite chitosan-reinforced scaffold fails to provide osteochondral regeneration. Int J Mol Sci. 2019;20:2227.
98. Schüttler KF, Götschenberg A, Klasan A, Stein T, Pehl A, Roessler PP, Figiel J, Heyse TJ, Efe T. Cell-free cartilage repair in large defects of the knee: increased failure rate 5 years after implantation of a collagen type I scaffold. Arch Orthop Trauma Surg. 2019;139(1):99–106.
99. Cipitria A, Reichert JC, Epari DR, Saifzadeh S, Berner A, Schell H, Mehta M, Schuetz MA, Duda GN, Hutmacher DW. Polycaprolactone scaffold and reduced rhBMP-7 dose for the regeneration of critical-sized defects in sheep tibiae. Biomaterials. 2013;34(38):9960–8.
100. Zhao X, Hu DA, Wu D, He F, Wang H, Huang L, Shi D, Liu Q, Ni N, Pakvasa M, Zhang Y, Fu K, Qin KH, Li AJ, Hagag O, Wang EJ, Sabharwal M, Wagstaff W, Reid RR, Lee MJ, Wolf JM, El Dafrawy M, Hynes K, Strelzow J, Ho SH, He TC, Athiviraham A. Applications of biocompatible scaffold materials in stem cell-based cartilage tissue engineering. Front Bioeng Biotechnol. 2021;9:603444.
101. Tanase CE, Popa MI, Verestiuc L. Biomimetic bone scaffolds based on chitosan and calcium phosphates. Mater Lett. 2011;65(11):1681–3.
102. Aprile P, Kelly DJ. Hydrostatic pressure regulates the volume, aggregation and chondrogenic differentiation of bone marrow derived stromal cells. Front Bioeng Biotechnol. 2020;8:619914.
103. Wang XY, Lu Y, Wang W, Wang QG, Liang J, Fan YJ, Zhang XD. 1 *in vitro* and *in vivo*. Regen Biomater. 2020;7(6):583–95.
104. Huang Y, Fan HQ, Gong XY, Yang L, Wang FY. Scaffold with natural calcified cartilage zone for osteochondral defect repair in minipigs. AJSM. 2021;49(7):1883–91.
105. Cai HX, Yao Y, Xu Y, Wang Q, Zou W, Liang J, Sun Y, Zhou CC, Fan YJ, Zhang XD. A Col I and BCP ceramic bi-layer scaffold implant promotes regeneration in osteochondral defects. RSC Adv. 2019;9(7):3740–8.
106. Wei XW, Liu BY, Liu G, Yang F, Cao F, Dou XJ, Yu WT, Wang BJ, Zheng GS, Cheng LL, Ma ZJ, Zhang Y, Yang JH, Wang ZH, Li JL, Cui DP, Wang W, Xie H, Li L, Zhang F, Lineaweaver WC, Zhao DW. Mesenchymal stem cell-loaded porous tantalum integrated with biomimetic 3D collagen-based scaffold to repair large osteochondral defects in goats. Stem Cell Res Ther. 2019;10:72.
107. Szwedowski D, Szczepanek J, Paczesny Ł, Pękała P, Zabrzyński J, Kruczyński J. Genetics in cartilage lesions: basic science and therapy approaches. Int J Mol Sci. 2020;21:5430.
108. Song H, Du HY, Li J, Wang MM, Wang JH, Ju XZ, Mu WD. Effect of fibroblast growth factor 2 on degenerative endplate chondrocyte: from anabolism to catabolism. Exp Mol Pathol. 2021;118:104590.
109. Sheehy EJ, Miller GJ, Amado I, Raftery RM, Chen G, Cortright K, Vazquez AG, O'Brien FJ. Mechanobiology-informed regenerative medicine: dose-controlled release of placental growth factor from a functionalized collagen-based scaffold promotes angiogenesis and accelerates bone defect healing. J Control Release. 2021;334:96–105.
110. Ayerst BI, Day AJ, Nurcombe V, Cool SM, Merry CL. New strategies for cartilage regeneration exploiting selected glycosaminoglycans to enhance cell fate determination. Biochem Soc Trans. 2014;42(3):703–9.
111. Gugjoo MB, Amarpal, Abdelbaset-Ismail A, Aithal HP, Kinjavdekar P, Kumar GS, Sharma GT. Allogeneic mesenchymal stem cells and growth factors in gel scaffold repair osteochondral defect in rabbit. Regen Med. 2020;15(2):1261–75.
112. Fang DP, Jin P, Huang QX, Yang Y, Zhao JM, Zheng L. Platelet-rich plasma promotes the regeneration of cartilage engineered by mesenchymal stem cells and collagen hydrogel via the TGF-beta/SMAD signaling pathway. J Cell Physiol. 2019;234(9):15627–37.
113. Amann E, Wolff P, Breel E, van Griensven M, Balmayor ER. Hyaluronic acid facilitates chondrogenesis and matrix deposition of human adipose derived mesenchymal stem cells and human chondrocytes co-cultures. Acta Biomater. 2017;52:130–44.
114. Liu QH, Wang J, Chen YP, Zhang ZP, Saunders L, Schipani E, Chen Q, Ma PX. Suppressing mesenchymal stem cell hypertrophy and endochondral ossification in 3D cartilage regeneration with nanofibrous poly(l-lactic acid) scaffold and matrilin-3. Acta Biomater. 2018;76:29–38.
115. Cheng MS, Yi X, Zhou Q. Overexpression of HIF-1alpha in bone marrow mesenchymal stem cells promote the repair of mandibular condylar osteochondral defect in a rabbit model. J Oral Maxillofac Surg. 2021;79(2):345(e1-e15).
116. Benya PD, Shaffer JD. Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell. 1982;30:215–24.
117. Francioli SE, Candrian C, Martin K, Heberer M, Martin I, Barbero A. Effect of three-dimensional expansion and cell seeding density on the cartilage-forming capacity of human articular chondrocytes in type II collagen sponges. J Biomed Mater Res A. 2010;95(3):924–31.
118. Chen YC, Liu HC, Lin FH, Chang CH. Development of engineered cartilage product from bone marrow mesenchymal stem cells: an example in Taiwan. J Musculoskel Res. 2021;24(01).
119. Raftery RM, Gonzalez Vazquez AG, Chen G, O'Brien FJ. Activation of the SOX-5, SOX-6, and SOX-9 trio of transcription factors using a gene-activated scaffold stimulates mesenchymal stromal cell chondrogenesis and inhibits endochondral ossification. Adv Healthc Mater. 2020;9:e1901827.
120. Bauza G, Pasto A, Mcculloch P, Lintner D, Brozovich A, Niclot FB, Khan I, Francis LW, Tasciotti E, Taraballi F. Improving the immunosuppressive potential of articular chondroprogenitors in a three-dimensional culture setting. Sci Rep. 2020;10:16610.
121. Kwon H, Haudenschild AK, Brown WE, Vapniarsky N, Paschos NK, Arzi B, Hu JC, Athanasiou KA. Tissue engineering potential of human dermis-isolated adult stem cells from multiple anatomical locations. PLoS ONE. 2017;12:0182531.
122. Donahue RP, Nordberg RC, Bielajew BJ, Hu JC, Athanasiou KA. The effect of neonatal, juvenile, and adult donors on rejuvenated neocartilage functional properties. Tissue Eng A. 2021.
123. Contentin R, Demoor M, Concari M, Desancé M, Audigié F, Branly T, Galéra P. Comparison of the chondrogenic potential of mesenchymal stem cells derived from bone marrow and umbilical cord blood intended for cartilage tissue engineering. Stem Cell Rev Rep. 2020;16(1):126–43.
124. Cao CX, Zhang YJ, Ye YQ, Sun TZ. Effects of cell phenotype and seeding density on the chondrogenic capacity of human osteoarthritic chondrocytes in type I collagen scaffolds. J Orthop Surg Res. 2020;15:120.
125. Scioli MG, Storti G, Bielli A, Sanchez M, Scimeca M, Gimble JM, Cervelli V, Orlandi A. CD146 expression regulates osteochondrogenic differentiation of human adipose-derived stem cells. J Cell Physiol. 2021;20:30506.
126. Cai HX, Wang PL, Xu Y, Yao Y, Liu J, Li T, Sun Y, Liang J, Fan YJ, Zhang XD. BMSCs-assisted injectable Col I hydrogel-regenerated cartilage defect by reconstructing superficial and calcified cartilage. Regen Biomater. 2020;7(1):35–45.
127. Yang JR, Li YQ, Liu YB, Li DX, Zhang L, Wang QG, Xiao YM, Zhang XD. Influence of hydrogel network microstructures on mesenchymal stem cell chondrogenesis *in vitro* and *in vivo*. Acta Biomater. 2019;91:159–72.
128. Brehm W, Aklin B, Yamashita T, Rieser F, Trüb T, Jakob RP, Mainil-Varlet P. Repair of superficial osteochondral defects with an autologous scaffold-free cartilage construct in a caprine model: implantation method and short-term results. Osteoarthr Cartilage. 2006;14(12):1214–26.

129. Kohane DS, Langer R. Ploymeric biomaterials in tissue engineering. Pediatr Res. 2008;63(5):487–91.
130. Zhu SC, Yuan QJ, Yin T, You J, Gu ZP, Xiong SB, Hu Y. Self-assemmbly of collagen-based biomaterials: preparation, characterizations and biomedical applications. J Mater Chem B. 2018;6:2650.
131. Schinagl RM, Gurskis D, Chen AC, Sah RL. Depth-dependent confined compression modulus fo full-thickness bovine articular cartilage. J Orthop Res. 1997;15:499–506.
132. Little CJ, Bawolin NK, Chen X. Mechanical properties of natural cartilage and tissue-engineered constructs. Tissue Eng B Rev. 2011;17(4):213–27.
133. Nguyen QT, Hwang Y, Chen AC, Varghese S, Sah RL. Cartilage-like mechanical properties of poly(ethylene glycol)-diacrylate hydrogels. Biomaterials. 2012;33(28):6682–90.
134. Yu XY, Li JL, Yang MT, Chen C, Munir S, You J, Yin T, Liu R, Xiong SB, Hu Y. Role of epigallocatechin gallate in collagen hydrogels modification based on physicochemical characterization and molecular docking. Food Chem. 2021;360:130068.
135. Suh DS, Yoo JC, Woo SH, Kwak AS. Intra-articular atelocollagen injection for the treatment of articular cartilage defects in rabbit model. Tissue Eng Regen Med. 2021;18(4):663–70.
136. Ling Y, Xu WK, Yang LF, Liang CY, Xu B. Improved the biocompatibility of cancellous bone with compound physicochemical decellularization process. Regen Biomater. 2020;7(5):443–51.
137. Rajagopal K, Ramesh S, Walter NM, Arora A, Katti DS, Madhuri V. *In vivo* cartilage regeneration in a multi-layered articular cartilage architecture mimicking scaffold. Bone Joint Res. 2020;9(9):601–12.
138. Saukko AEA, Nykänen O, Sarin JK, Nissi MJ, te Moller NCR, Weinans H, Mancini IAD, Visser J, Brommer H, van Weeren PR, Malda J, Grinstaff MW, Töyräs J. Dual-contrast computed tomography enables detection of equine posttraumatic osteoarthritis *in vitro*. J Orthop Res. 2021.
139. Mainil-Varlet P, Van Damme B, Nesic D, Knutsen G, Kandel R, Roberts S. A new histology scoring system for the assessment of the quality of human cartilage repair: ICRS II. Am J Sports Med. 2010;38(5):880–90.
140. Guerin G, Pujol N. Repair of large condylar osteochondral defects of the knee by collagen scaffold. Minimum two-year outcomes. Orthop Traumatol Surg Res. 2020;106(3):475–9.
141. Miyahira MKC, Novaretti JV, Astur DC, Kaleka CC, Amaro JT, Cohen M. Larger chondral lesions treated with collagen membrane—matrix-induced autologous chondrogenesis—show larger increase in clinical scores. Revista Brasileira Ortopedia. 2021;56(3):333–9.
142. Kayaalp ME, Cirdi YU, Kopf S, Becker R. Prone-positioned knee arthroscopy for isolated retropatellar cartilage defects with gel-type autologous chondrocyte implantation. Oper Orthop Traumatol. 2021;33(5):436–44.
143. Puhakka J, Paatela T, Salonius E, Muhonen V, Meller A, Vasara A, Kautiainen H, Kosola J, Kiviranta I. Arthroscopic international cartilage repair society classification system has only moderate reliability in a porcine cartilage repair model. Am J Sports Med. 2021;49(6):1524–9.
144. Bouyer M, Garot C, Machillot P, Vollaire J, Fitzpatrick V, Morand S, Boutonnat J, Josserand V, Bettega G, Picart C. 3D-printed scaffold combined to 2D osteoinductive coatings to repair a critical-size mandibular bone defect. Mater Today Bio. 2021;11:100113.
145. Peterson L, Brittberg M, Kiviranta I, Akerlund EL, Lindahl A. Autologous chondrocytes transplantation biomechanics and long-term durability. Am J Sport Med. 2002;30(1):2–12.

Publisher's Note