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Review article

Advancements in hydrogel design for articular cartilage regeneration: A comprehensive review

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ABSTRACT

This review paper explores the cutting-edge advancements in hydrogel design for articular cartilage regeneration (CR). Articular cartilage (AC) defects are a common occurrence worldwide that can lead to joint breakdown at a later stage of the disease, necessitating immediate intervention to prevent progressive degeneration of cartilage. Decades of research into the biomedical applications of hydrogels have revealed their tremendous potential, particularly in soft tissue engineering, including CR. Hydrogels are highly tunable and can be designed to meet the key criteria needed for a template in CR. This paper aims to identify those criteria, including the hydrogel components, mechanical properties, biodegradability, structural design, and integration capability with the adjacent native tissue and delves into the benefits that CR can obtain through appropriate design. Stratified-structural hydrogels that emulate the native cartilage structure, as well as the impact of environmental stimuli on the regeneration outcome, have also been discussed. By examining recent advances and emerging techniques, this paper offers valuable insights into developing effective hydrogel-based therapies for AC repair.

1. Introduction

Articular cartilage (AC) is a highly specialized tissue that plays a crucial role in joint functions by providing a low-friction, load-bearing surface [1]. Unfortunately, due to its limited intrinsic regenerative capacity, cartilage lesions caused by trauma or aging often lead to osteoarthritis and are accompanied by pain, functional impairment, and a reduced quality of life for patients. Although both palliative and surgical treatments are available to address cartilage defects, they have not yet been shown to be capable of completely regenerating healthy cartilage in the affected joint [1].

In recent years, tissue engineering techniques for cartilage have emerged as promising alternatives to overcome the limitations of traditional treatments. These innovative approaches utilize novel engineering and biological methods to accelerate the development of neotissue to replace damaged tissue. Due to its complex nature and high water content, AC repair requires a biomaterial matrix that possesses similar viscoelastic properties [2].

Hydrogels, three-dimensional (3D) networks of hydrophilic polymers, have emerged as attractive scaffolds for cartilage tissue engineering due to their unique properties, including their biocompatibility, tunable mechanical characteristics, and excellent permeability for crucial elements such as oxygen, nutrients, and water-soluble metabolites [3]. Hydrogels play a pivotal role in preserving the rounded

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morphology and phenotype of chondrocytes or stromal cells while also establishing an optimal 3D local microenvironment that fosters interactions among cells and networks [4,5]. Understanding the interactions between hydrogels and encapsulated cells is complex. In addition to soluble signals, cells can also sense their milieu through mechanical and physical cues, such as the rigidity of the extracellular matrix (ECM) nearby [6].

Depending on the cross-linking chemistry, the manufactured hydrogel could be either implantable or injectable (Fig. 1) [7,8]. Implantable hydrogel scaffolds offer significant clinical benefits, including high mechanical strength and customizable shapes. These scaffolds, pre-fabricated using methods like 3D printing techniques (extrusion or direct ink writing (DIW)), are surgically implanted at the target site, demonstrating significant potential in therapeutic applications [8]. Injectable hydrogels, thanks to their sol-gel properties, can easily fill cartilage defects. These hydrogels, responsive to temperature or shear force, ensure proper filling of irregular knee joint shapes, even cell distribution, and enable minimally invasive surgeries [9,10] (Table 1). Hydrogels have the advantage of being adjustable and their properties can be tuned depending on the application.

Key parameters including type of materials, mechanical strength, matrix stiffness and elasticity, biodegradability, integrity, porosity, and interconnectivity are crucial in regulating cell fate, particularly in terms of proliferation and differentiation. This opens a large area of investigation to determine how these factors individually or in combination with one another, contribute to better reconstruction of the tissue. However, there is a paucity of studies addressing the essential processing parameters in this domain. Current review papers provide a different perspective on the research of hydrogels in this field [14,15]. The primary objective of this review is to showcase the most recent advancements in the critical factors involved in designing hydrogels for CR. Hydrogel components, mechanical properties, biodegradability, structural design, and integration capability with the adjacent native tissue are the hydrogel design parameters that are covered in the following sections. The paper begins with a thorough overview of the current knowledge in the field, highlighting the challenges and limitations of existing hydrogel-based cartilage tissue engineering approaches. Section 2 explores the recent advancements and breakthroughs in hydrogel design, and section 3 explores stratified-structural hydrogels that mimic the native cartilage architecture. This is followed by a section that discusses environmental stimuli and how they are engineered to modulate cellular responses and tissue regeneration by elucidating the interplay between hydrogel structures. Fig. 2 provides a comprehensive summary of the entire review's content. This paper aims to underscore that biomimetic hydrogels can restore the composition, structure, and biomechanical functions of articular cartilage. We conclude by sharing our perspective on the future of hydrogel design for joint cartilage restoration. Such advancements hold significant promise in addressing the yet unmet clinical needs for cartilage tissue engineering and improvement in the quality of life for individuals suffering from cartilage-related disorders, thus directly impacting the United Nations's Sustainable Development Goal 3.

Table 1
A comparative analysis of various hydrogels.

Type of hydrogel	Properties	References
Implantable	- Customization - Easily adjustable shape - High mechanical strength - Suitable for load-bearing tissue	[8,11,12]
Injectable	- Minimally invasive - Suitable for irregularly shaped defects - Reduces the need for extensive surgical procedures - Adjustable rheological characteristics	[9,13]

2. Hydrogel design parameters

Designing hydrogels with specific characteristics and functions is a complex task that requires meticulous manipulation of various factors. For cartilage regeneration (CR), the primary focus should be on selecting the appropriate hydrogel components, as the material composition directly influences the hydrogel's properties and performance. The mechanical properties of the hydrogel scaffold must align with the target tissue to ensure proper support and functionality [16]. Tuning stiffness, elasticity, and load-bearing capacity is crucial for developing a material that mimics natural cartilage, enabling effective load distribution, tissue integration, and functional restoration. Integration with native tissue ensures immediate function and long-term stability of restored cartilage. Biodegradability is another key factor, as the hydrogel must degrade at an optimal rate to maintain structural integrity while allowing cell infiltration and tissue integration. Rapid degradation can compromise support, while slow degradation may impede cell infiltration and trigger foreign body reactions. Achieving the right degradation rate is essential for successful tissue engineering. Additionally, developing porous and interconnected structures within hydrogels is vital for cellular infiltration, nutrient diffusion, and waste removal. This supports cell growth

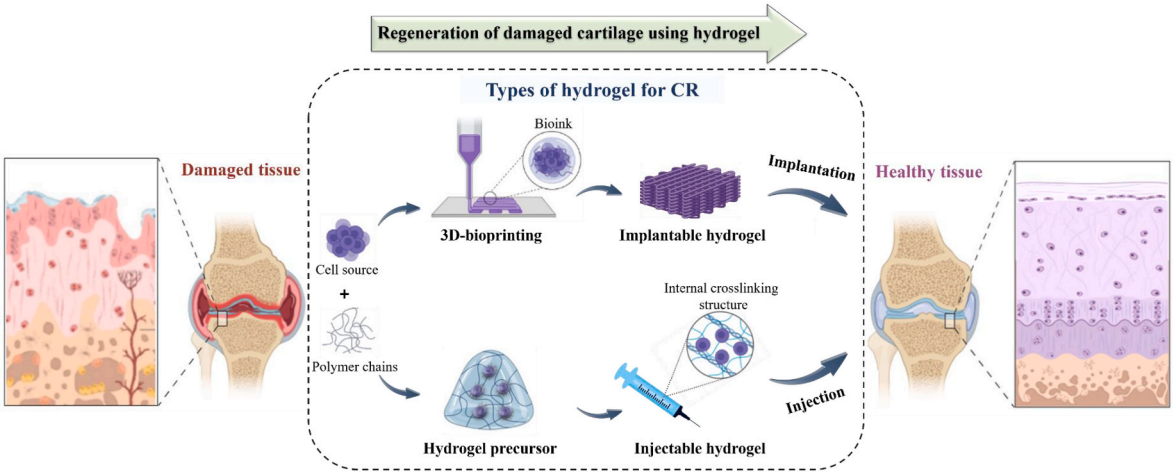

Fig. 1. Schematic illustration of approach employing hydrogels in cartilage tissue regeneration and different hydrogels designed to be placed at the injury site within the knee joint. Implantable hydrogel scaffolds produced via 3D-bioprinting that provide a structured support for tissue regeneration. Injectable hydrogels that can be administered directly into the injury site.

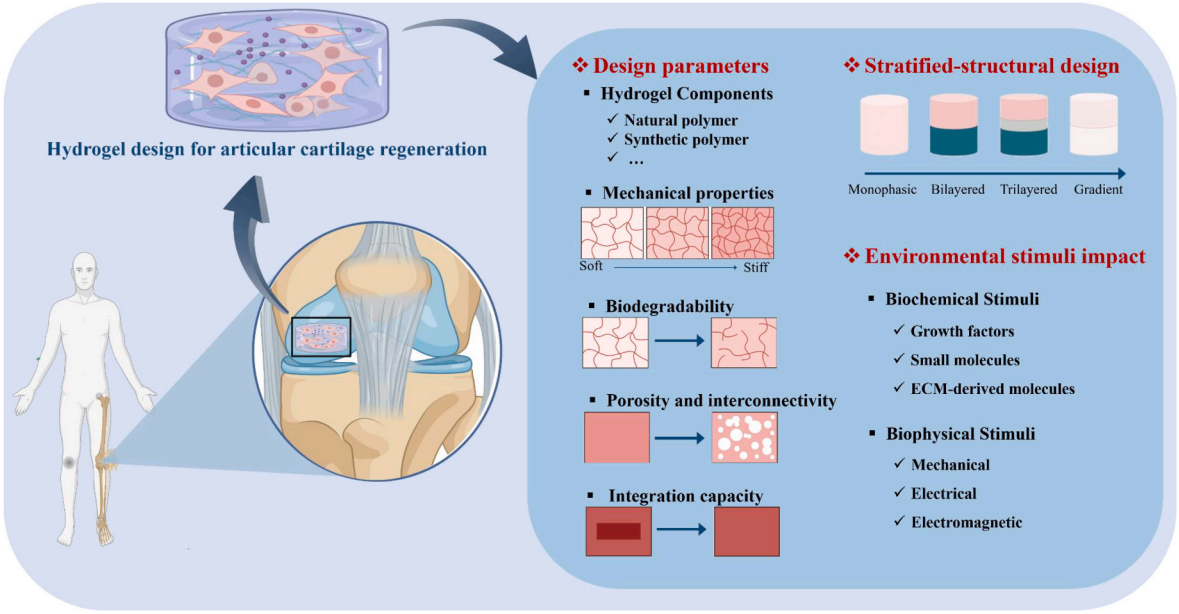

Fig. 2. Schematic illustration of key parameters in designing hydrogels for CR, including hydrogel components, mechanical properties, biodegradability, porosity and interconnectivity, and integration with adjacent native tissue. Additionally, it highlights other essential factors such as stratified-structural hydrogels and the influence of environmental stimuli.

and functionality within the hydrogel. In the following section, we provide a detailed overview of these parameters and illustrate their impact on cell-hydrogel interactions. By considering these significant factors, researchers can optimize hydrogel design to meet the specific requirements for cartilage repair.

2.1. Hydrogel components

Biomaterials, including natural and synthetic polymers, have been widely employed as the foundational elements of hydrogels in cartilage tissue engineering (Table 2). While this review does not delve deeply into these biomaterials, it does highlight some of the most commonly used and contemporary materials. Natural hydrogels, composed of collagen (Col), hyaluronic acid (HA), alginate (Alg), silk fibroin (SF), gelatin (Gel), chitosan (Ch), chondroitin sulfate (CS), and dextran (DEX), are highly valued for their inherent biocompatibility, environmental sensitivity, and abundance [17–20]. They also contain degradation moieties like hydrolyzable ester and enzyme-mediated hydrolytic amide groups, providing natural binding sites for cell interaction. However, they suffer from low stability, poor mechanical properties, and rapid degradation [2,14]. In contrast, synthetic hydrogels such as poly (lactic-co-glycolic acid) (PLGA), polylactide acid (PLA), poly (vinyl alcohol) (PVA), poly (ethylene glycol) (PEG), Poly (N-isopropylacrylamide) (PNiPAAm), and polycaprolactone (PCL) offer tunable chemistry, allowing optimization of physicochemical and mechanical properties [21–25]. They can be designed with various

Table 2
A summary of hydrogels with different polymer types and their features for CR.

	Biomaterial	Other components	Cell type	Strength (kPa)	Degradation (day)	Pores (μm)	References
Natural	Col	HA	rBMSCs	~12	14	–	[20]
		SF	RBMSCs	8	≥2	–	[27]
	HA	Col short nanofibers, Ch	rBMSCs	9.54	≥30	10–65	[19]
		Col	RBMSCs	~700	≥7 (*in vivo*)	–	[28]
	Alg	HA, icariin, extracellular vesicles	rBMSCs	319.6	≥11	–	[18]
		Ch, PVA	–	141000	–	2.5	[29]
	SF	RGD, DNA	rBMSCs	–	28	35.1	[17]
		Gel	RBMSCs	4980	–	22.01	[30]
	Gel	Glucosamine	Rabbit chondrocytes	–	≥10	50–200	[31]
		BC, LAP	Auricular chondrocytes	~1400	–	172.8	[32]
	Ch	GelMA	–	169.3	1	–	[33]
		Alg-nHap, HA	hMSCs	108.33	–	156	[34]
	CS	Hydroxypropyl chitin	rBMSCs	~6	≥25	–	[35]
	SF	SF	Human chondrocyte	100	~7	143	[36]
	DEX	HA- tyramine	hMSCs, bCHs	3.2	–	–	[37]
Synthetic	PLGA	PEG, Gel, TGF-β1	hDPSCs	16000	≥21	202.05	[21]
	PLA	GelMA, autologous auricle cartilage	–	~50	≥18		[22]
	PVA	Ch, graphene oxide	L929 mouse fibroblasts	2150	–	7.86	[23]
	PEG	PLGA	rBMSCs	–	≥35	–	[38]
		KGN, Ch	PB-MSCs	1620	≥42	202.69	[39]
	PNiPAAm	CS, DEX	Human articular chondrocyte	–	≥21	68	[24]
	PCL	β-CD-modified Alg/cartilage ECM, KGN, starch	hADSCs	17200	≥30	300	[25]

Abbreviations: GelMA: methacrylated gelatin, TGF-β: transforming growth factor-β, rBMSCs: rat bone marrow MSCs, RGD: arginine-glycine-aspartic acid, RBMSCs: rabbit BMSCs, nHap: nanohydroxyapatite, hMSCs: human MSCs, bCHs: bovine chondrocytes, hDPSCs: human dental pulp stem cells, β-CD: beta cyclodextrin, KGN: kartogenin, hADSCs: human adipose-derived stem cells, LAP: lithium phenyl-2,4,6-trimethylbenzoylphosphinate, PB-MSCs: peripheral blood MSCs.

molecular weights, block structures, and degradable linkages to meet specific mechanical and degradation requirements. Despite these advantages, synthetic hydrogels lack adhesion sites, biocompatibility, and produce undesirable degradation products [26]. Indeed, combining natural and synthetic biomaterials can leverage the strengths of both types. This synergy can result in hydrogel scaffolds that are both functional and adaptable for cartilage tissue engineering [15].

To ensure bioactivity and biocompatibility in practical applications, polymers used for hydrogels can be sourced from endogenous substances within organisms. These include glycosaminoglycans, macromolecular proteins, and DNA nucleotides. Zhao et al. introduced DNA-SF DN hydrogels with adjustable surface stiffness [40]. These hydrogels, formed through DNA base-pairing, promote β-sheet structure formation by constraining and aggregating SF molecules. The second network was established through enzyme-mediated SF cross-linking. It was found that DNA-SF DN hydrogels were highly effective in promoting chondrogenic differentiation. Building on this, Shen et al. developed RGD-SF-DNA hydrogel microspheres (RSD-MSs) capable of loading BMSCs [17]. Initially, microfluidic technology, silk methacrylate, and supramolecular DNA hydrogel techniques were used to create SF-DNA DN hydrogel microspheres (SD-MSs). These microspheres were then surface-modified via photopolymerization with Pep-RGDfKA. The results indicated that RSD-MSs are ideal for fabricating and maintaining cartilage organoids long-term, offering an innovative strategy and biomaterial choice for CR (Fig. 3A).

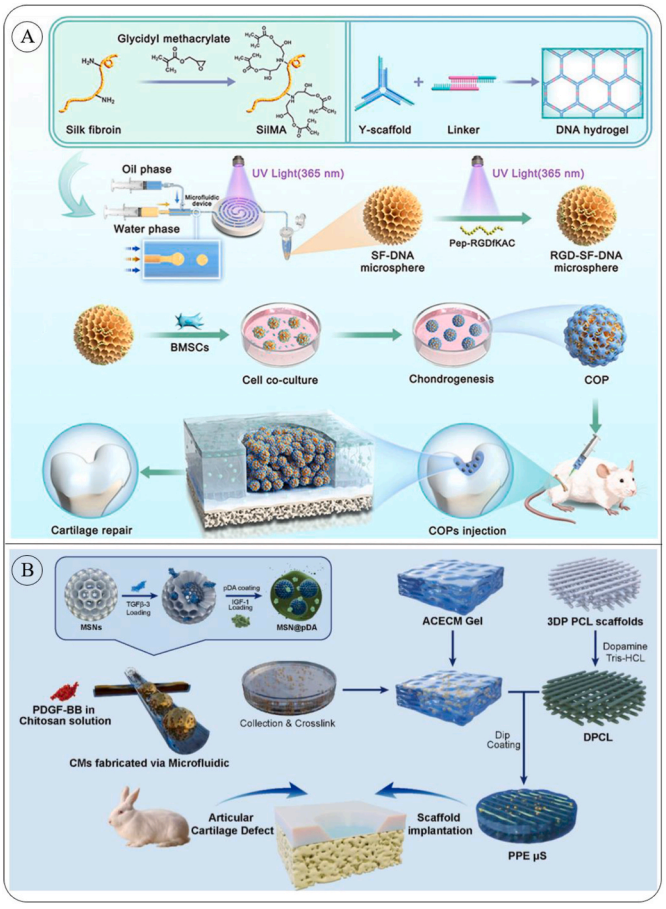

Fig. 3. A) Schematic illustration of the synthesis of RGD-SF-DNA microspheres and their use in creating cartilage organoid precursor and CR. Reproduced under the terms of the CC BY-NC-ND 4.0 license [17]. Copyright 2024, The Authors, published by Elsevier. B) Schematic illustration of a cartilage lacuna-biomimetic hydrogel scaffold for multifunctional CR, featuring TGFβ3-loaded MSN@pDA microspheres with IGF-1 and PDGF-BB, fabricated using microfluidic technology. Reproduced under the terms of the CC BY-NC-ND 4.0 license [41]. Copyright 2024, The Authors, published by Elsevier.

Recent advancements have led to the development of novel bioinspired hydrogels. For instance, hydrogels enhanced with polydopamine (pDA) improve cell adhesion and tissue integrity. Li et al. developed a composite microsphere system for CR by integrating TGF-β3 into mesoporous silica nanoparticles (MSNs) and encapsulating them with insulin-like growth factor-1 (IGF-1) in pDA microspheres [41]. These were blended with a Ch hydrogel containing platelet-derived growth factor-BB (PDGF-BB) using microfluidic technology. The pDA reduced initial inflammation, while the sustained release of GFs promoted stem cell attraction, chondrogenesis, and matrix formation. The microspheres were incorporated into an acellular cartilage extracellular matrix and combined with a pDA-modified PCL scaffold, enhancing cartilage repair and protection (Fig. 3A). Additionally, those incorporating sugar-based additives like Manuka honey offer antibacterial properties and enhance mechanical and biological characteristics [42]. Aloe vera gel, enriched with glycoprotein fractions, has also demonstrated a significant chondrogenic effect [43].

2.2. Mechanical properties of hydrogels

2.2.1. Mechanical strength

Native AC possesses unique mechanical properties, including a specific range of compressive strength, elastic modulus, and viscoelastic behavior [16,44]. The mechanical characteristics of the hydrogel scaffold play a crucial role in providing appropriate support, load distribution, and functionality, which are all essential for successful CR [45–47]. Several studies have focused on tuning the mechanical properties of hydrogels to mimic those of the native cartilage. AC is both stiff and tough, unlike conventional hydrogels which deform under mechanical forces and lack the necessary properties for cartilage repair [48]. More often a stiffening process is achieved at the expense of lowering the capability of adsorbing and holding gel water, which can compromise the embedded cell viability by impeding cell migration and biomolecule diffusion. Additionally, their brittleness inhibits force distribution, reducing lubrication functionality. However, parameters like pore size, swelling ratio, strength, and toughness can be improved without compromising stiffness [49,50]. Gan et al. developed a hydrogel by incorporating dopamine methacrylate oligomers into GelMA, resulting in two key mechanical advantages over single-network GelMA hydrogels [51]. First, the increased distance between GelMA chains enhanced the hydrogel's failure strain. Second, dopamine created a secondary cross-linking network that dissipated energy through physical sacrificial bonds under load (Fig. 4A). Current efforts focus on developing tough hydrogels that maintain stiffness and distribute energy to prevent force concentration in damaged zone [52,53]. A promising approach in cartilage repair involves using multiple polymers and cross-linking mechanisms, each serving a specific purpose [54].

Double network (DN) hydrogels, introduced by Gong's group in 2003, enhance mechanical properties by combining a brittle, densely cross-linked polymer with a soft, loosely cross-linked one [56,57]. After several studies examining the potential candidates in cartilage repair, they reached the conclusion that poly (2-acrylamide-2-methyl propane sulfonic acid)/poly (N, N_0-dimethyl acrylamide) (PAMPS/PDMAAm) prepared in two steps in the existence of initiator and UV lamps is the most promising one. Furthermore, the negatively charged PAMPS resemble the abundant proteoglycans (PGs) in articular cartilage, making the hydrogel a bioactive material for chondrogenesis. Yasuda et al. demonstrated that the chondrogenesis ability of (PAMPS/PDMAAm) hydrogel in terms of high expression of collagen II (Col II), aggrecan (Agc), and SRY-Box transcription factor9 (Sox9) when implanted in the rabbit's femoral groove was much higher compared to PVA hydrogel [58]. Still, several factors must be taken into account when designing a DN hydrogel serving as an ideal cell carrier in CR. First, if the first network is permanently covalent, which means it is not able to recover

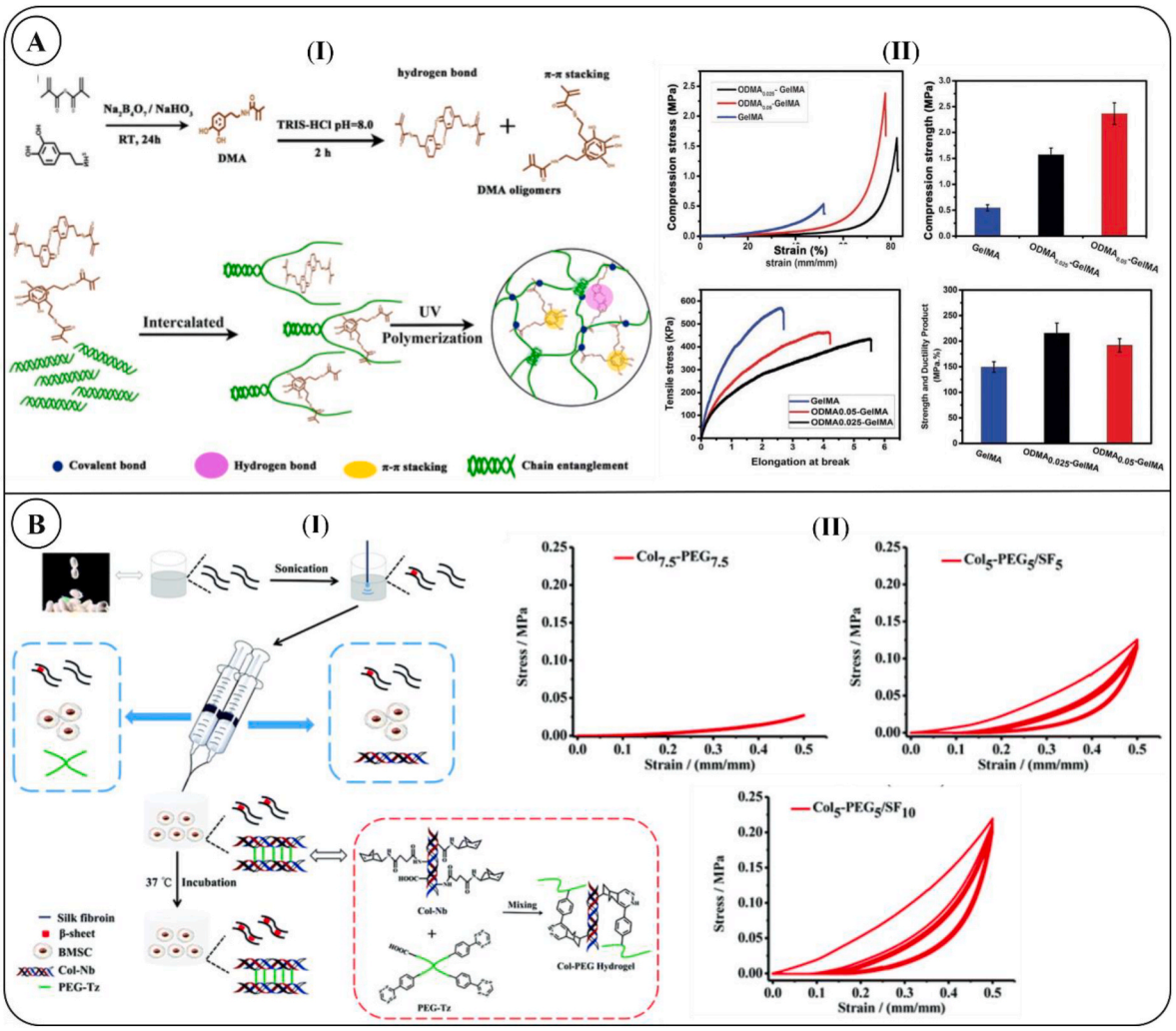

Fig. 4. A) (I) Synthesis of dopamine methacrylate and its oligomers (ODMA). (II) Mechanical behavior of the ODMA–GelMA hydrogel. Reproduced with permission [51]. Copyright 2018, Royal Society of Chemistry. B) (I) Formation of injectable PEG-Col/SF DN network by covalent cross-linking of PEG-Tz and Col-Nb. (II) Cyclic compression stress–strain curves of hydrogels and compression-recovery of the Col5-PEG5/SF5 hydrogel. Reproduced with permission [55]. Copyright 2021, Royal Society of Chemistry.

after removing the load, the utilization of this kind of hydrogel in articular cartilage, which encounters millions of cycling loads, will be restrained [56]. Webber et al. observed significant hysteresis in PAMPS/PDMAAm hydrogels with two covalently cross-linked networks under compression [59]. In subsequent cycles, the loading curve matched the unloading curve of the previous cycle, indicating that the brittle cross-linking bonds could not revert to their original state after dissipating energy. To address this, the first network could use dynamic covalent or supramolecular bonds that recover after energy dissipation [60]. Zhou et al. developed a DN hydrogel with a first network formed by the Schiff reaction between Gel and oxidized dextran (DEX), and a second network of GelMA under UV illumination [61]. This dynamic covalent bond resulted in higher energy loss and significant hysteresis after five cycles compared to a single network GelMA hydrogel. However, full recovery was not achieved unless the first network could rapidly and almost completely return to its original state. Supramolecular interactions, which act as sacrificial bonds, can distribute energy during loading and recover to their original state after the force is removed. Zhang et al. developed an injectable DN hydrogel for cartilage repair, composed of Col-norbornene (Col-Nb), tetrazine-modified polyethylene glycol (PEG-Tz), and SF [55]. The first network, Col-PEG, was formed by rapid cross-linking of bioorthogonal groups, norbornene and tetrazine. The second network consisted of SF β-sheets cross-linked by ultrasonication. This Col-PEG/SF hydrogel demonstrated superior compression resistance and recovery compared to the Col-PEG hydrogel (Fig. 4B). The combination of polyacrylamide (PAAm) and ionically cross-linked Alg marked a breakthrough in DN hydrogels achieving high stiffness (~1 MPa) and super high fracture energy (~16 kJ m^{-2}) [62]. This network, responsible for self-recovery, showed varying behavior based on storage medium and resting time. Maximum recovery (74 % of initial toughness) was achieved after one day in 80 °C mineral oil [63]. However, this did not meet the fatigue-resistant conditions needed for CR. Recent studies have reduced recovery time by using iron (III) ions and citrate solution, achieving full recovery in 3 min [64]. A new hydrogel with hydrophobic ionic monomers and UV illumination showed complete recovery in 2 h [65]. Another hydrogel with PAM and self-assembling peptide fibers demonstrated rapid self-recovery, high fracture energy (~2670 J m^{-2}), and high strength (~1.6 MPa), suitable for CR. Chemically cross-linking the second network to the first improved self-recovery efficiency [66]. Second, DN hydrogels used in cartilage face durability challenges when ionic cross-link comprises one or both networks. Traces of mobile ions are present in synovial fluid [67] that is in direct contact with articular cartilage, exchanging its ions with those in cross-linked hydrogel, thus jeopardizing the stability of the implanted hydrogel [56]. Third, despite mechanical advances in photoinduced hydrogels like methacrylate and acrylate derivatives,

their harsh preparation environments limit their use as 3D cell-encapsulated materials for cartilage defects. Sequential polymerization can leave toxic components that threaten cell viability [68]. A mixture of PEG and poly (ethylene oxide)-methacrylate forms a gel under low-intensity UV light (2–3 mW cm^{-2}), which can trap and support chondrocytes. After two weeks, the chondrocytes produce a new matrix rich in PGs, essential for cartilage structure and function. Although the cell-hydrogel mixture reached a stiffness of 70 kPa after six weeks of static incubation, similar to natural cartilage, its initial stiffness was only 1 kPa, much lower than *in vivo* constructs for cartilage repair [69]. The stiffening observed in static culture may not occur *in vivo*, where the construct faces various forces that could cause rupture. Efforts to balance UV intensity and cytotoxicity have led to more reliable methods like Schiff-base reactions [61,70] and Michael addition, reducing gel time to 20 min in a cell-friendly environment [71].

Combining toughness and injectability in hydrogels has garnered significant interest. Reversible interactions that enable self-healing at the microscopic level can be incorporated into multi-cross-linking hydrogels at the macroscopic level, resulting in injectable hydrogels with similar properties [60]. Rodell et al. created an injectable hydrogel using guest-host (GH) interactions by incorporating β-cyclodextrin and adamantane into methacrylated hyaluronic acid (MeHA), achieving a toughness of 13 kJ m^{-3}. GH interactions involve a host molecule binding a guest molecule inside its cavity [71]. DN tough hydrogel bioinks, composed of GelMA, o-nitrobenzyl (NB)-grafted hyaluronic acid (HA-NB), and elastin, were developed for bioprinting complex elastic tissues. These GHE hydrogels exhibited high toughness (~45 kJ m^{-3}), stretchability (~170 % strain), elasticity, anti-fatigue ability, viscoelasticity, and resilience [72]. However, there is still room for improvement, as sheep cartilage toughness is around 110 kJ m^{-3} [73].

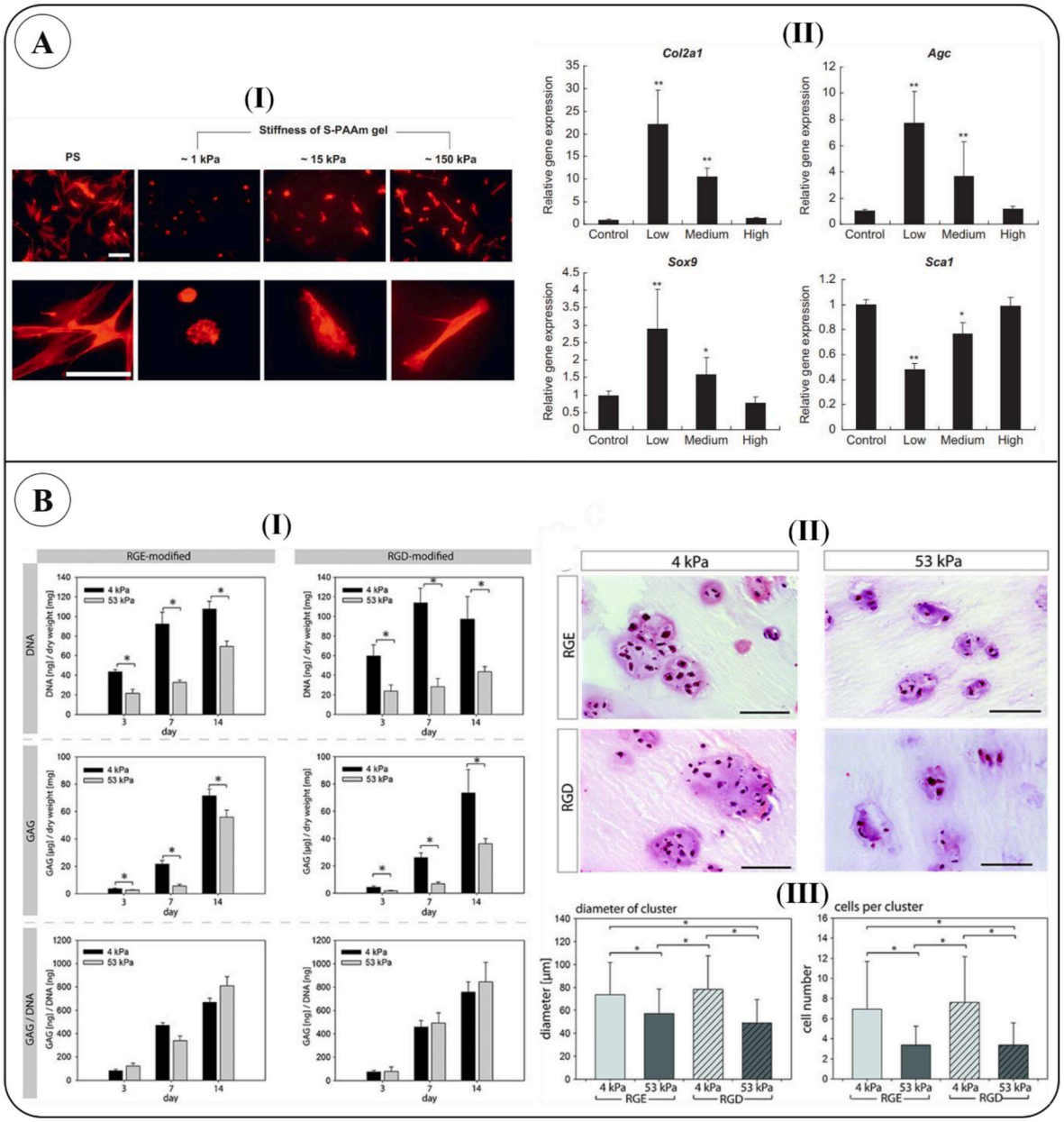

Fig. 5. A) (I) F-actin structures in MSCs on S-PAAm gels with different stiffness. (II) Gene expression of Col 2a1, Agc, Sox9 and Sca1 in MSCs after 1 week on PS (control) and S-PAAm gels. Reproduced with permission [79]. Copyright 2013, Elsevier Ltd. B) (I) DNA and GAG quantification. (II) H&E stain of day 14 sections. (III) Cell cluster size, and Cell number per cluster. Reproduced with permission [81]. Copyright 2011, John Wiley and Sons.

2.2.2. Stiffness and elasticity

Substrate elasticity can determine stem cell fate or lineage commitment [74]. On stiffer substrates, MSCs [75], fibroblasts [76], and endothelial cells [77] have all shown improved cell adherence, spreading and proliferation. As previously indicated, natural gels are not ideal for studying the mechanosensing of chondrocytes to substrates that require precisely controlled stiffness because of their limited ability to adjust mechanical properties. By changing the type of cross-link, the concentration ratio of composite materials, external stimulation, molecular weight and adding nanoparticles (NPs), researchers created stiffness-tunable hydrogels [78]. Kwon and Yasuda developed sulfonate-coated polyacrylamide (S-PAAm) gels with elastic moduli of 1, 15, and 150 kPa. MSCs cultured on high-stiffness gels exhibited strong stress fiber expression, while those on low-stiffness gels had round shapes with more cortical actin. Notably, lower stiffness gels led to higher mRNA levels of chondrogenic markers (Col 2a1, Agc, Sox9) even without differentiation supplements (Fig. 5A) [79]. Lin et al. created hydrogels with varying mechanical properties by adjusting the molecular weight of PEG-diacrylate (PEGDA) precursors from 3.4 to 20 kDa [80]. Higher molecular weight PEGDA increased the hydrogel's swelling ratio and mesh size. PEGDA-6 kDa and −10 kDa hydrogels had the highest glycosaminoglycan (GAG) content at week four, while PEGDA-20 kDa had greater Col content. PEGDA-10 kDa showed the highest overexpression of Col II. Schuh et al. investigated the effects of substrate stiffness, adhesion site density, and porosity independently using agarose hydrogels modified with RGD [81]. Cells retained the chondrogenic phenotype regardless of the stiffness of the substrate or the availability of adhesion sites. The amount of GAG and DNA in softer gels modified with RGD and RGE (arginyl-glycyl-glutamyl) was found to be significantly higher, as determined by quantification. However, the amount of GAG per DNA was unaffected by the stiffness of the substrate or the availability of adhesion sites. The average diameter of cell ECM clusters in softer gels was significantly larger than in stiffer gels, according to hematoxylin and eosin (H&E) staining (Fig. 5B).

For a variety of regenerative medicine applications, flexible methods for selectively manipulating the biomaterial microenvironment are required to find synergies between biochemical and mechanical cues [82]. Park et al. demonstrated that matrix stiffness and GFs—two significant stimuli in the cellular microenvironment—have an impact on MSC differentiation [83]. When MSCs were grown on Col gels as opposed to stiff substrates, Col II rose significantly. Col II expression was further elevated by TGF-β on Col gels, but not on stiff substrates. Separating the effects of matrix elasticity and biochemical signals on cells is challenging due to the unique properties of natural materials [84]. Chen et al. studied chondrocyte mechanoresponses to external forces, biochemical variables, and substrate elasticity using polyacrylamide hydrogels (1, 11, and 90 kPa) treated with TGF-β1 [85]. TGF-β1 enhanced chondrocyte responses, increasing traction force, cellular stiffness, and expression of Col II and Agc, especially on stiffer substrates.

2.2.3. Load-bearing capacity

AC is a flexible tissue that covers bone ends in joints, enabling smooth, low-friction movements and distributing mechanical loads. Hydrogels are promising for CR due to their water-rich, soft nature. However, they must have high load-bearing capacity and low deformation under stress to match cartilage performance [86,87]. Reinforcing hydrogels with fibers or other components can enhance their strength and toughness. Fiber-reinforced hydrogels also reduce joint friction and wear by providing lubrication and cushioning [88]. AC has a complex structure, primarily composed of cartilage-specific Cols and charged PGs, with varying compositions across different regions and developmental stages [89]. Advanced imaging techniques, such as light, confocal, and electron microscopy, reveal distinct compartments within the cartilage matrix: the pericellular matrix (PCM), territorial matrix (TM), and interterritorial matrix (ITM) [90]. The PCM, lacking fibrillar Col, mainly consists of PGs and concentrated Col VI, which stabilizes Cols, PGs, and glycoproteins and interacts with cell receptors to initiate signaling pathways [91]. The TM is characterized by Col fibrils and increased PGs in the interfibrillar space. The ITM has a lower density of unevenly distributed Col fibrils, with interfibrillar PGs and link protein (LP) combined with HA. Matrix vesicles in the ITM play a role in calcification and mineralization within the ECM [92].

Winter et al. used a tracer to study the assembly of newly synthesized Agc in the PCM before its transport to the ECM. Agc forms complexes with HA, providing cartilage with load-bearing properties [93,94]. The PCM facilitates these complexes' formation and influences the mechanical environment of chondrocytes by affecting fluid flow and load transmission, protecting them from excessive compression [92]. Williamson et al. found that Col content increases while CS content decreases in bovine AC during development, improving compressive properties [95,96]. The ECM's amount and distribution affect cartilage's mechanical properties, becoming functional when connecting the neo-matrix associated with each cell. Matrix proteins and PGs are slowly deposited and remodeled in adult cartilage, with PG deposition more restricted to the PCM early in culture and higher matrix protein deposition outside the PCM later [97–99].

Mauck et al. showed that chondrocytes cultured in agarose hydrogels with intermittent loading and higher seeding densities had stronger material properties than those without loading [100]. They found no significant differences in PG and Col content between different loaded and unloaded hydrogels, suggesting that loading may affect material properties by changing the structure, the production of small ECM molecules or the size of PG aggregates. Erickson et al. showed that increasing the MSC seeding density in low-concentration HA constructs increased the biomechanical properties [101]. They hypothesized that this was due to a shorter diffusion distance for the matrix production. In Table 3, a summary of hydrogels' investigations with various mechanical properties is provided.

2.3. Biodegradability

The other noteworthy property of hydrogels is their degradability pattern, which influences the cross-link density and biomechanics of hydrogels and the behavior of cells. Hydrogels need to degrade gradually and be metabolized by the body to support tissue regeneration and avoid long-term implant issues. A continuous network enhances the biochemical and biomechanical properties of tissue constructs. However, in cartilage tissue engineering, matrix deposition often occurs only near the cells, creating isolated cartilage islands within the hydrogel [118]. To improve matrix distribution in hydrogels, two main strategies can be employed: The first solution is to add specific segments or organic biopolymers that degrade enzymatically or hydrolytically, allowing large ECM molecules like Col to diffuse through the network [119]. The second solution is to use viscoelastic hydrogels that mimic the mechanical properties of natural cartilage [120]. These hydrogels reduce forces through mechanical yielding and matrix remodeling, promoting larger areas of cartilage matrix formation per chondrocyte [118]. Sridhar et al. theorized that local ECM "pockets" can connect before the bulk hydrogel reaches reverse gelation, provided the weak pericellular regions overlap. However, if hydrogel degradation is too slow, it impedes neo-tissue growth, while too rapid degradation can lead to loss of mechanical integrity [119,121,122].

To control degradation rates in line with matrix production, cell-mediated degradation was developed. Bahney et al. studied gene expression of matrix metalloproteinases (MMP) and ADAMTS during MSC chondrogenesis in semi-interpenetrating networks PEGDA-based hydrogel [122]. They found that MMP-7, expressed by encapsulated hMSCs, had a temporal profile matching chondrogenesis. By embedding specific peptide substrates (MMP-7 substrates) into the polymer backbone, they created enzymatically degradable hydrogels. This improved the intercellular distribution of the Col II matrix and increased the

Table 3
Summary of hydrogels' investigations with various mechanical properties and crosslinking methods in CR.

Biomaterial	Crosslinking approach	Mechanical properties	Effects on cell behaviors	References
PEGDMA, CS, HA, and HS	Photo-crosslinking	• Adjusting the matrix stiffness (3, 30, and 90 kPa)	• Increasing expression of chondrogenic genes in soft hydrogels (3 kPa) with increasing HA concentration	[102]
PDMS		• Stiffness Range: 1.4–135 kPa	• Increased chondrocyte phenotype and higher expression of Col II at 6 kPa stiffness	[103]
GelMA, and iron oxide NPs (Fe_2O_3)	Chemical	• Adjusts stiffness of Fe_2O_3/GelMA hybrid hydrogel by varying the concentration of MNPs (1.30–1.80 MPa)	• Modulates chondrocyte properties • Enhances mitochondrial oxidative phosphorylation and lipid catabolism • Validates hybrid hydrogel's regenerative potential in a rat cartilage defect model	[104]
Alg	Physical ($CaCl_2$) and chemical	• DC-Alg hydrogels have significantly higher mechanical properties than singly cross-linked hydrogels, with a maximum compressive stress of 1600 kPa and a compressive modulus of 3400 kPa	• Expresses chondrogenic genes • Develops cartilage tissue	[105]
PDLLA, PEG, and HA	Microwave irradiation	• Over 4 weeks, constructs with HA and TGF-β see their Young's modulus rise to 400 kPa	• Adding HA and TGF-β to *in vitro* cultures increases sulfated GAG production	[106]
BC and SF	Physical	• The BC/SF hydrogel has a higher compressive strength (1.49 MPa at 55.21 % strain) and better shape recovery than pure BC hydrogel	• The blended scaffold enhances cell viability and alignment	[107]
FGMA	Photo-crosslinking (UV)	• The compression modulus of FGMAs increases with higher MA substitution • 4 % FGMA3 has a modulus 41 times higher than GelMA (230.80 kPa) • FGMA3 shows higher compression strength at maximum strain compared to GelMA (1035.00 kPa)	• FGMA hydrogels support favorable cell viability • After 8 weeks, the compressive stiffness of regenerated cartilage tissue is similar to that of normal cartilage	[108]
Acrylamide-acrylic acid and SNPs	Chemical, and physical	• Adding 0.6 wt% of SNPs increases the hydrogel's compressive strength to 1.4 MPa and doubles its elastic modulus to 240 kPa • Samples can withstand up to 85 % strain without breaking • All nanocomposite hydrogels show improved viscoelastic responses by halving stress • Improved stress relaxation is observed due to the enhanced elastic modulus	• A 570 μm thick lubricious layer with uniform funnel-like porosity can be created	[109]
4-arm star PEG	Chemical (vinyl sulfone and short dithiol)	• Compressive strength ~20 MPa	• Chondrocytes encapsulated in hydrogel show proliferation and abundant ECM production in subcutaneous culture in SCID mice	[110]
Alg, CS, and Sr	Physical ($SrCl_2$)	• Tunable stiffness in the range of 50–120 kPa by changing the $SrCl_2$ concentration	• High compatibility with cells • Positive effect on the inhibition of apoptosis	[111]
PVA, and PGF	Physical (PGF)	• Improvement of mechanical properties of hydrogel with maximum tensile strength of 12.44 MPa and Young's modulus of 68.35 MPa	• Improvement of cell adhesion and proliferation with PGF	[112]
HA, Col I, and Col II	Photo-crosslinking (blue light)	• Significant reduction of compressive and dynamic modulus of hydrogel scaffolds by incorporating Col II (~8 kPa, 16 kPa)	• High biocompatibility of hydrogels • Increase sGAG deposition in chondrogenic culture	[20]
$MASO_3$	Radical polymerization (BAP, MBAA)	• Compressive moduli from 2.5 0.1 to 1708.7 67.7 kPa	-	[113]
PEG and LP NPs	Chemical (Butane diamine)	• Increasing storage and loss modulus due to the addition of LP NPs • High mechanical properties with a storage modulus of about 500 kPa without LP addition	• Biocompatibility of hydrogels (cell viability is more than 80 %)	[114]
GE and PSBMA	Chemical cross-linking and transesterification	• Excellent compressive stress at approximately 3.50 MPa and low loss factors (0.023–0.049)	• Safe for cells, and excellent lubricity	[115]
PVA, nHap, HACC, Ch	Dual physically cross-linked (simply freezing/thawing technique and an immersing process)	• Fracture tensile stress (2.70 ± 0.24 MPa), toughness (14.09 ± 2.06 MJ/m^3) and compressive modulus (0.88 ± 0.09 MPa)	• Excellent cytocompatibility because of the existence of nHA	[116]
PVA, glycerol, and BC	-	• Compressive modulus: 19.56 MPa; friction coefficient: 0.057 at joint-equivalent load and low sliding speed	-	[117]

Abbreviations: HS: heparan sulfate, PDMS: polydimethylsiloxane, DC-Alg: double cross-linked alginate, sGAG: sulfated glycosaminoglycan, PDLLA: poly-d, l-lactic acid, BC: bacterial cellulose, FGMA: methacrylated flaxseed gum, SNPs: silica NPs, Sr: strontium, PGF: phosphate glass fiber, $MASO_3$: 3-sulfopropylmethacrylate potassium, BAP: N, N_0-Bis-(acryloyl)-piperazin, MBAA: N, N′-methylenebis(acrylamide), LP: laponite, GE: glycerol ethoxylate, PSBMA: zwitterionic poly-sulfobetaine methacrylate, HACC: 2-hydroxypropyltrimethyl ammonium chloride.

dynamic modulus in neo-cartilage constructs. Parmar et al. developed degradable Col-mimetic hydrogels using HA or CS-binding peptides, cross-linked with an MMP7-sensitive peptide. The hydrogel with CS bind showed the greatest improvement in AC deposition/degradation ratio and significantly influenced MMP7 gene expression and activity. They further enhanced the hydrogels by adding peptide sequences that bound heparin, integrin, and HA, along with a mix of MMP7 and aggrecanase cleavable peptides. This combination significantly improved hMSC chondrogenesis and maintained mechanical integrity during the culture period [123,124].

By using ester linkage in polymers such as PLA or PGA in various MW, distribution, crystallinity and scaffold porosity, the degradation of hydrogels could be controlled [125,126]. Bryant et al. designed photo-encapsulated hydrogels with chondrocytes by incorporating

degradable triblock copolymer PEG-LA-DA into PEGDMA. The fastest degradable group, containing 85 % PEG-LA-DA, showed significantly higher total Col content throughout the neo-tissue [127]. To address challenges in scaffold degradation synchronization and cell adhesion, a novel microcavity gel (MCG) model was developed. This model, inspired by the dynamic outgrowth of chondrocytes at the gel edge, facilitates cell proliferation and ECM secretion through multiple gel edges and microspherical cavities, termed Phase Transfer Cell Culture (PTCC) [128]. Fan et al. compared TPT-MCG with conventional TPT-DA-based hydrogels and found that the micro-cavitary structure accelerated degradation, benefiting cell proliferation and cartilage-specific ECM production. After 21 days, cell density in TPT-MCG constructs was 5.6 times higher than in TPT-G constructs, with increased total collagen and GAG content [129].

Bryant et al. developed PEG hydrogels with varying compressive moduli and incorporated PLA to enhance ECM molecule dispersion. They found an optimal cross-linking density that balances mesh size, water content, and gel mechanics for cell viability [130]. Tho et al. created hyaluronic acid-tyramine hydrogels with tunable properties and observed that lower cross-linked samples supported higher cell density and sGAG biosynthesis, with cells showing a chondrocyte-like appearance after 21 days [131].

Natural hydrogels are gaining attention for their biodegradability and biocompatibility. However, Ch-based hydrogels often suffer from poor mechanical strength and elasticity. Shen et al. addressed this by creating Ch-Gel hydrogels through in situ precipitation, achieving mechanical properties comparable to or better than human cartilage [132]. These hydrogels showed 65.9 % degradation in 70 days, aligning with synthetic CR rates. They also demonstrated a compressive toughness of approximately 75.8 J m^{-2} and superior adhesion and proliferation of human thyroid cartilage cells. The pore size varied, with Ch hydrogels having 180–300 μm pores, while those with 4 % Gel had pores around 100 μm.

2.4. Porosity and interconnectivity

Developing porous and interconnected structures within hydrogels is crucial for cellular infiltration, nutrient diffusion, waste removal, and functional tissue formation. Although the ideal pore size for polymer scaffolds is debated, cartilage scaffolds typically have over 70 % porosity with interconnected pores between 100 and 500 μm [133]. The best scaffold balances interconnectivity and mechanical stability, ensuring enough void volume to support chondrocyte adaptation while maintaining strength, as higher porosity reduces mechanical strength [134, 135].

Optimizing porosity and interconnectivity in hydrogel scaffolds can be achieved through various methods. One approach is sacrificial templating, where a material is incorporated into the hydrogel and later

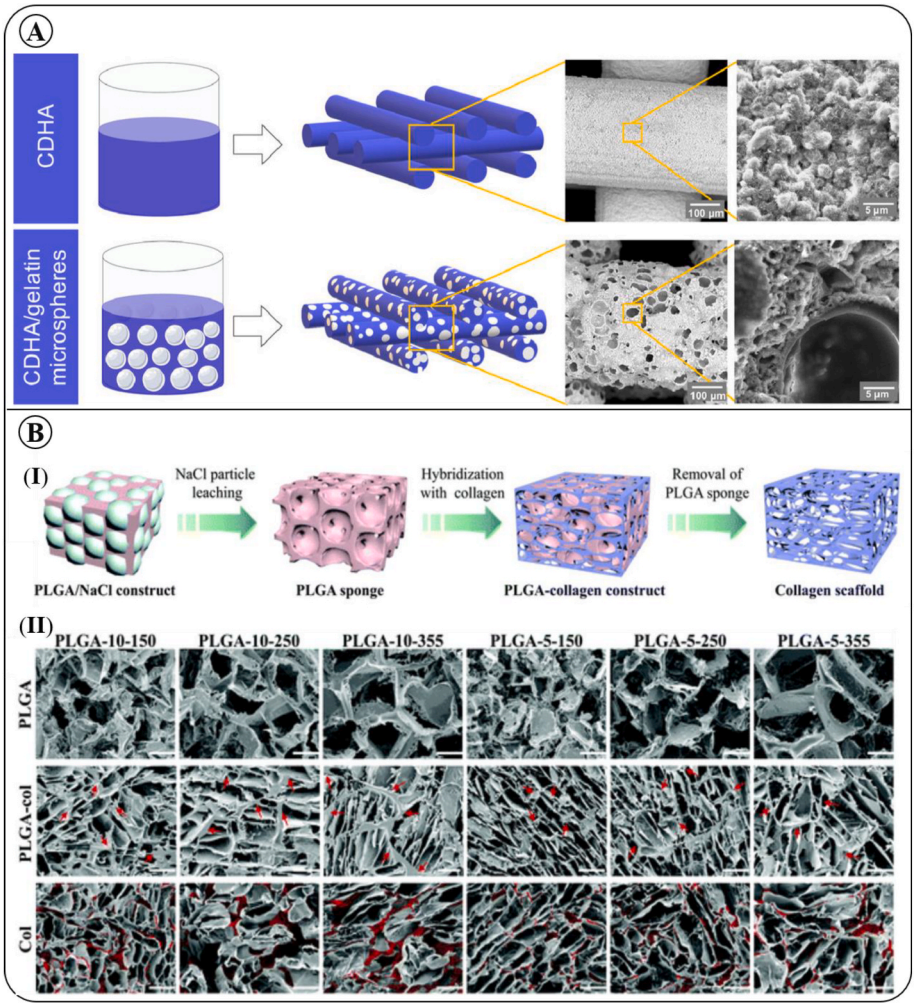

Fig. 6. A) Developing hierarchical porous biomimetic hydroxyapatite scaffolds using DIW technique. (I) incorporating Gel microspheres as sacrificial templates, and SEM images of the filaments, showing the concave pores after Gel microspheres dissolve partially. Reproduced under the terms of the CC BY-NC-ND 4.0 license [136]. Copyright 2021, Elsevier Ltd. B) (I) Schematic illustration of interconnected Col porous scaffolds prepared with sacrificial PLGA sponge templates. (II) SEM images of PLGA templates, PLGA–Col constructs and Col scaffolds cross-sections. Reproduced with permission [137]. Copyright 2021, Royal Society of Chemistry.

removed to create pores. For example, Konka et al. used Gel microspheres in hydroxyapatite scaffolds, resulting in a porous structure suitable for tissue engineering (Fig. 6A) [136]. Another method is particulate leaching, where water-soluble particles are dispersed in the hydrogel and then dissolved to form pores. Additionally, PLGA sponges were used as templates to create Col scaffolds with interconnected pores. These scaffolds supported uniform chondrocyte distribution, rapid cell division, and increased cartilaginous gene expression and extracellular matrix secretion. The most effective results were seen with Col scaffolds templated by PLGA sponges with a high weight ratio and large salt particles (Fig. 6B) [137].

Various other methods, including 3D-printing [51], lyophilization [138], gas foaming [139], polymer phase separation [140], and emulsified droplets, or polymer microspheres [141] have been developed to create macroporous structures. Among the diverse array of 3D bioprinting strategies, extrusion-based printing, inkjet-based printing, and laser-based bioprinting are the most commonly used (Fig. 7). Each method has its own strengths and challenges, such as cost, accuracy, and time efficiency. The primary distinction between these standard 3D bioprinting techniques lies in their ability to precisely control the microstructure geometry [142,143]. Before printing, a CAD software system designs the 3D models and sets the parameters. Different inks and methods require specific parameters. The hydrogel ink is mixed and placed in the ink storage box, then applied layer by layer to the building platform by the 3D-printer. Each layer hardens before the next is added, gradually building up the 3D hydrogel precursor, which is then linked together [144,145]. However, printing soft biological materials in the air can lead to deformation and loss of shape. To address this, the Free Reversible Embedding of Suspended Hydrogels (FRESH) technique prints bioinks within a yield-stress support bath, which holds the bioinks in place until they cure [146]. This method involves embedding the printed hydrogel within a secondary hydrogel that acts as temporary, thermoreversible, and biocompatible support [147]. Table 4 shows a summary of hydrogels' investigations with different pore sizes for CR.

Different stages of tissue development require varying macropore densities and sizes. Low porosity provides structural stability and preserves transplanted cells, while increased porosity over time aids nutrient diffusion, cell proliferation, and matrix formation [155]. Zhang et al. developed a bilayered 3D-printed scaffold with a swelling-dependent gate, featuring a lower layer with smaller pores and an upper matrix with larger pores. This design allows bone marrow MSCs to infiltrate initially and later block excess blood support, aiding stable chondrogenesis (Fig. 8A) [156]. Biodegradable polymers can create scaffolds that increase porosity over time, but controlling the timing and degree of macropore formation remains challenging [157]. Existing methods often expose cells to harsh conditions, reducing survival rates. Seeding cells on prefabricated macroporous scaffolds can result in low efficiency and uneven distribution [158]. Encapsulating cells with non-cytotoxic porogens offers better control over cell distribution [159]. Han et al. used stimuli-responsive porogens to create dynamic hydrogels with tunable macropores, responding to stimuli like temperature, chelation, and enzymatic digestion [160].

Manipulating pore sizes in hydrogels can be achieved through various techniques, such as modifying polymer concentration, adjusting gelation conditions, and using rapid freeze-drying [162]. Li et al. showed that changing the concentration of PVA can regulate pore size, with an initial increase followed by a decrease as the concentration rises [163]. Lyophilized Alg hydrogel can significantly increase pore size to 200–300 μm [164]. Cross-linking density within the hydrogel network is another method to adjust pore size and porosity [165]. Cross-linking agents form covalent bonds between polymer chains, affecting the hydrogel's structure. By varying the concentration of these agents, researchers can control pore size and distribution. Yen et al. found that increasing methacrylate modification in BSAGMA (bovine serum albumin glycidyl methacrylate) hydrogels reduced pore sizes [166]. Wang et al. created a multifunctional polycitrate-based hydrogel (PCCGA) that retained its porous structure after freeze-drying, with pore size decreasing as concentration increased (Fig. 8B) [161]. In contrast, PEGDA hydrogel had fewer pores and more cracks due to lower cross-linking density. A hydrogel with lower cross-linking density absorbs more water, resulting in larger pore sizes [167].

2.5. Integration capacity

Integrating implants with native tissue is crucial for immediate functionality and long-term performance of repaired cartilage [168]. A strong interface between a scaffold and biological surface ensures a uniform arrangement of new tissue [169]. Conventional treatments like auto and allografts and microfracture often result in poor lateral integration with surrounding tissue [170]. Integrating hydrogels into

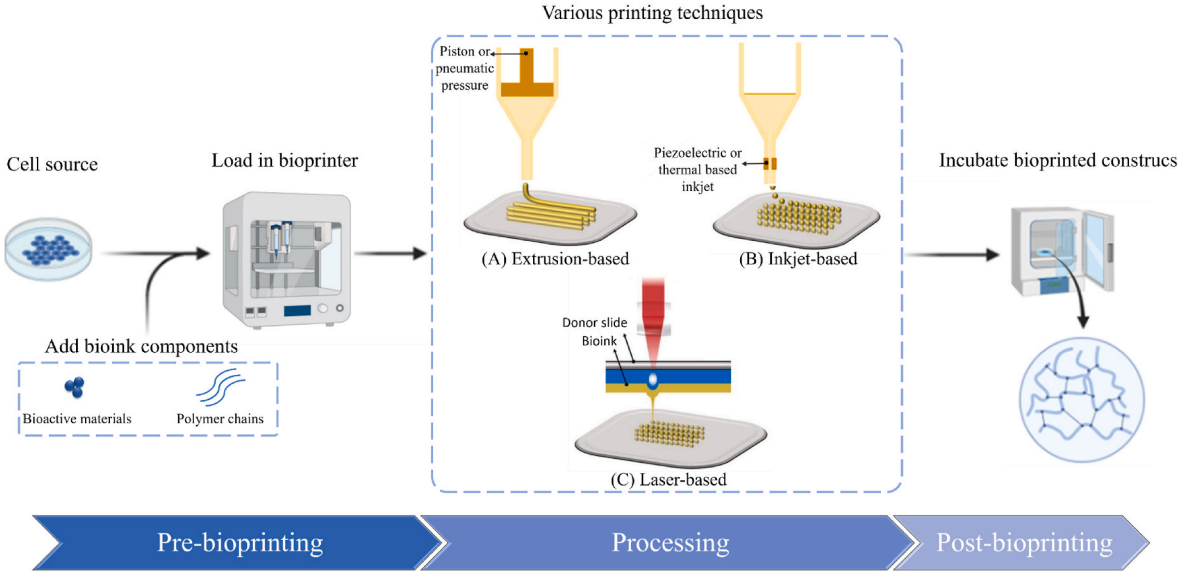

Fig. 7. Schematic illustration of the steps for bioprinting and different printing methods. (A) An extrusion printer dispensing cell-loaded solutions or hydrogels by air pressure or hand force, (B) an inkjet printer drops small bioink droplets to build a tissue layer by layer, and (C) a laser-assisted printer transfers the bioink to the substrate as droplets with a laser pulse.

Table 4
Summary of hydrogels' investigations with different pore sizes and porosity for CR.

Method	Biomaterial	Crosslinking approach	Pore size	Porosity (%)	Reference
Porogen leaching (sucrose particles)	Gel, HA, and Alg	Chemical (EDAC), physical ($CaCl_2$)	100 μm	70	[148]
3D printing (extrusion)	b-TPUe, and PCL	–	1.5 mm	73	[149]
Porogen leaching (NaCl particles)	PCL	–	100–150 μm	–	[150]
Lyophilization	SF, and Col	Physical (ethanol)	112 μm	89	[151]
Lyophilization	Col II, Col I, and HA	Chemical (EDAC/NHS)	155 μm	99	[152]
Porogen leaching (NaCl particles)	PEG	Photo-crosslinking with UV	15–82 μm	30	[153]
3D printing (extrusion)	Silica, PTHF, and PCL	–	210 μm	42	[154]

Abbreviations: EDAC: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, b-TPUe: 1,4-butanediol thermoplastic polyurethane, PTHF: poly(tetrahydrofuran), NHS: N-hydroxysuccinimide.

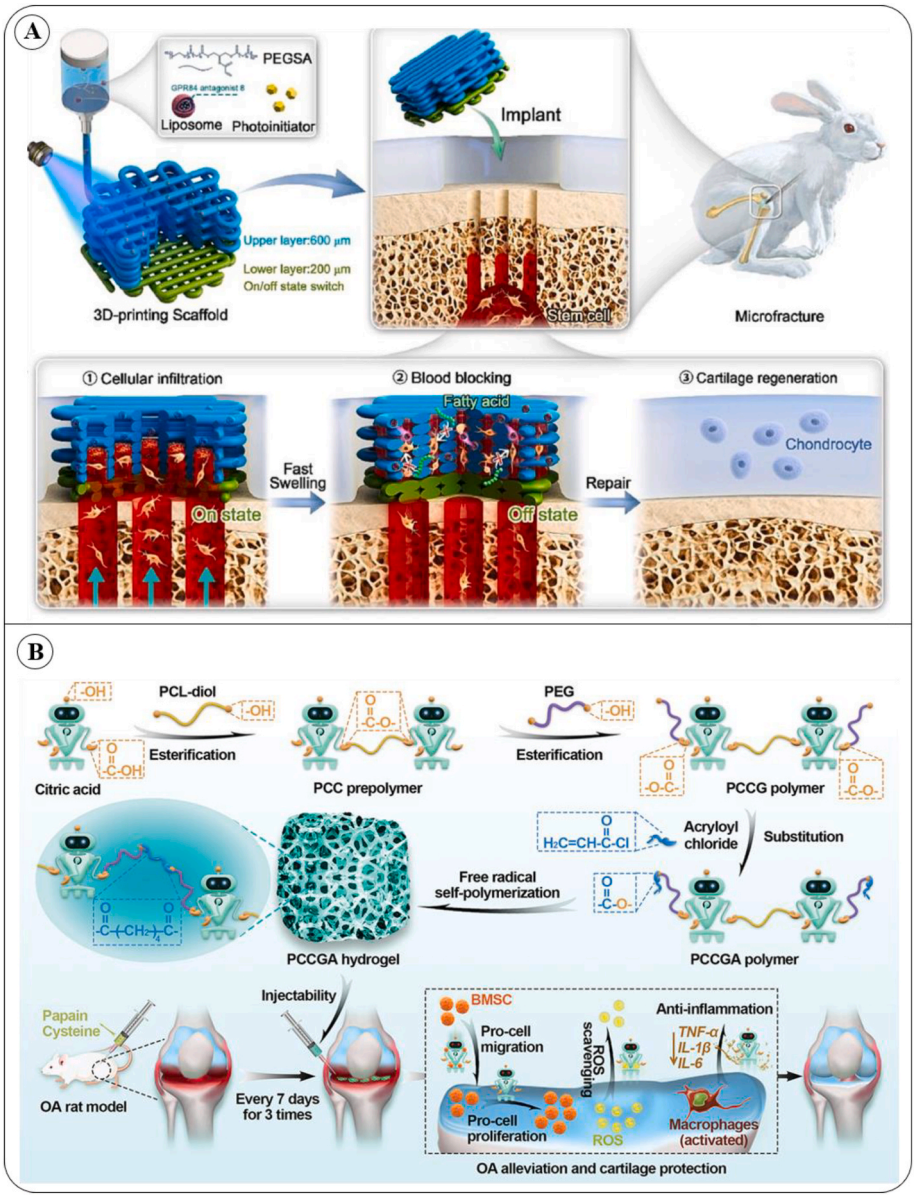

Fig. 8. A) Schematic illustration of bilayered scaffolds with two pore sizes for cartilage repair. Post-implantation, they swell from bone marrow blood, allowing BMSC infiltration before pore closure, while the upper layer houses BMSCs. Reproduced with permission [156]. Copyright 2024, Elsevier B.V. B) Schematic illustration of multifunctional PCCGA hydrogel design for OA alleviation and cartilage protection. Reproduced with permission [161]. Copyright 2023, John Wiley and Sons.

cartilage is particularly challenging due to the slippery nature of dense ECM and the mechanically harsh joint environment, making cartilage bonding difficult [171,172]. While integration with host bone is more achievable, inadequate adhesion between hydrogels and biological surfaces limits their biomedical applications [173].

Integrating neo-cartilage with natural cartilage includes using sutures and bioadhesives like fibrin glue [174]. However, fibrin glue degrades quickly, especially with chondrocytes [175], prompting research

into more durable bioadhesives that can withstand joint mechanical forces and provide long-term integration [168,176–180]. Hydrogel-tissue integration offers secure biological fixation and reduces infection risks, protecting neo-tissue and aiding the repair process [169]. Studies show that lack of integration can lead to the failure of cartilage repair [168,181,182].

One challenge in cartilage repair is cell death at the interface between the host and repair cartilage, as seen in treatments like autologous chondrocyte implantation [183]. Using inhibitors of necrosis (Nec-1) and apoptosis (ZVF) in the culture medium can reduce cell death [184,185]. Increasing cellular density at the interface, either by repopulating the scaffold with chondrocytes or attracting adjacent cells, can also help [186]. Dense ECM in AC hinders cell migration, for this reason, through chemical modification of biomaterials with cell-attracting components such as heparin [187] or intercalating oligomer of dopamine methacrylamide (ODMA) [188], enzymatic treatment of cartilage removing the Col networks and PGs barriers [189,190] or implanting hydrogels synergically with further microfracture creating a pathway for cell invasion and deposition [171], researchers stimulate this crucial cellular migration for achieving better integration. Even with adequate cell density in the interface zone, the environment must allow for proper diffusion of newly synthesized Col, which is crucial for integration [191]. Researchers can achieve this by selecting an appropriate macromer concentration when designing hydrogels. Erickson et al. found that seeding MSCs in methacrylated HA at a lower concentration (1 %) led to the strongest integration, likely due to increased Col production and better fiber contribution [192].

Injectable hydrogels, known for their ease of administration and stimuli-responsiveness, facilitate integration with surrounding cartilage by evenly dispersing cells and biomolecules and filling irregular tissue defects [193–195]. Tailoring the gel time is crucial for strong integration; it should be neither too long nor too short to prevent material flow or compromised integration [176,196]. Injectable hydrogels are also used for complex diseases like osteoarthritis, with shear-thinning properties preventing cell sedimentation [197,198]. Hou et al. incorporated Ureido-pyrimidinone (UPy) units into DEX polymer, creating a shear-thinning hydrogel that integrates two hydrogels embedded with chondrocytes and BMSCs within minutes due to dynamic hydrogen bonding (Fig. 9A) [199].

The importance of hydrogel-cartilage integration is twofold. In a cell-free approach, seamless integration allows adjacent cells to migrate into the defect site. In a cell-laden approach, strong adhesion ensures the hydrogel retains embedded cells and their derivatives effectively during healing [201]. One promising method is targeting cartilage matrix proteins for covalent binding with chemically modified hydrogels. Teixeira et al. used horseradish peroxidase to incorporate tyramine into

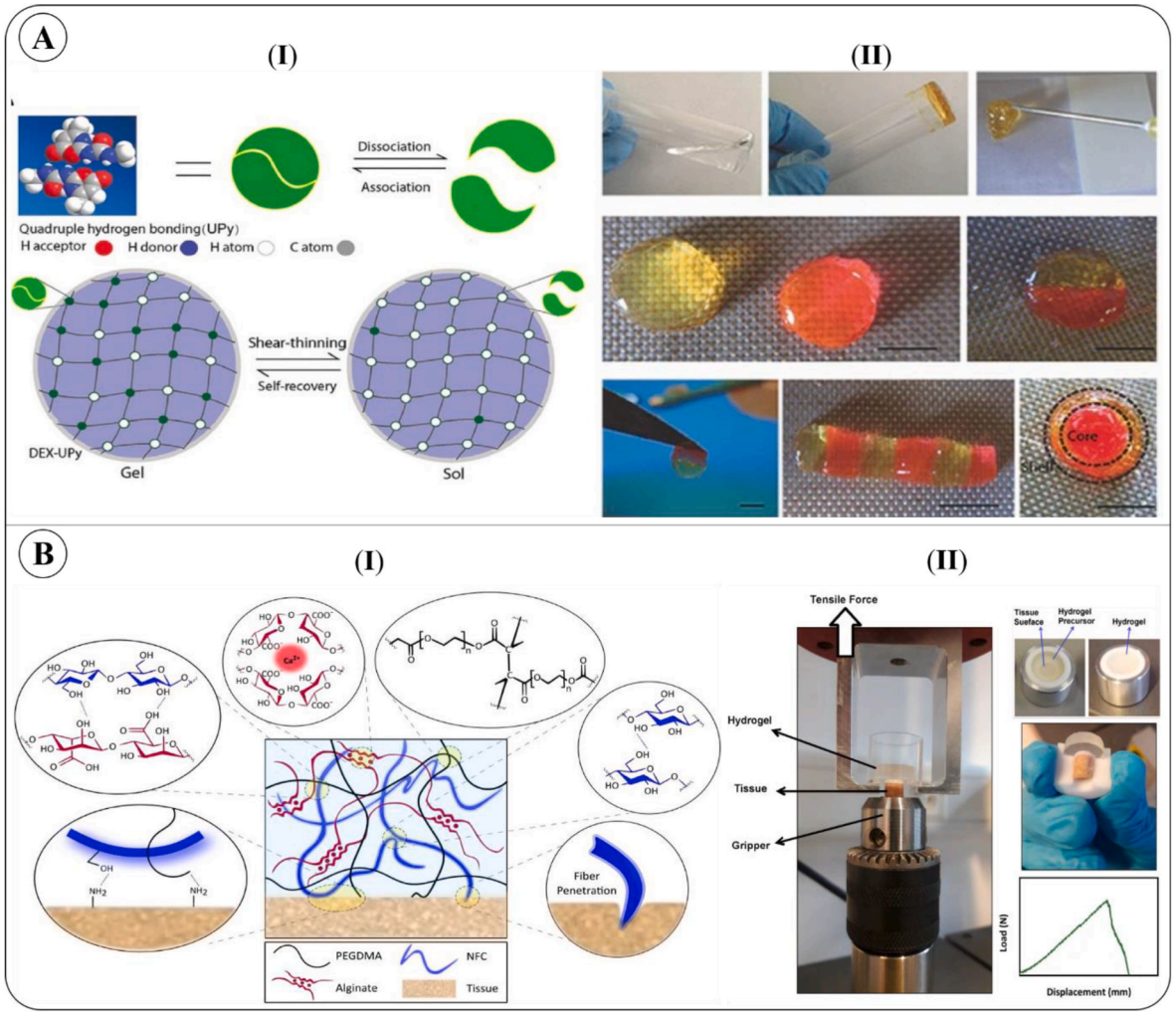

Fig. 9. A) Design of a DEX-UPy hydrogel for multi-tissue complex regeneration. (I) schematic illustration of DEX-UPy hydrogel formation and the mechanisms of the shear-thinning and self-recovery properties. (II) Formation of DEX-UPy hydrogel, its injectability and self-integrating property. Reproduced with permission [199]. Copyright 2015, Wiley-VCH. B) Schematic illustration of the adhesive design of the hydrogel. (I) The dissipative matrix consists of PEGDMA interpenetrated with an ionically crosslinked natural polymer (Alg), reinforced by cellulose fibers. (II) The custom-made adhesion setup for evaluating hydrogel-tissue attachment in traction mode. Reproduced (adapted) with permission [200]. Copyright 2018, American Chemical Society.

DEX polymer, enabling the hydrogel to bind with tyrosine residues in cartilage. Increasing tyramine residues strengthened the adhesion between the hydrogel and surrounding tissue, enhancing the role of covalent binding in adhesion strength [187].

Wang et al. developed a photo-initiated hydrogel that covalently integrates into cartilage Col networks [189]. After removing surface PGs with chondroitinase ABC to expose Col, tyrosine residues are oxidized and then exposed to UV light, initiating a reaction with PEGDA. This hydrogel showed strong interface strength in mechanical torsion tests, similar to pure gel, while unoxidized cartilage constructs slipped under small torsional strain. Strehin et al. used a similar mechanism to develop a CS-based hydrogel that binds to proteins in cartilage [176]. The strength of this hydrogel depends on pH; higher pH reduces gelation time, leading to fewer amide bonds and a softer hydrogel. At neutral pH, the hydrogel's adhesion strength was ten times stronger than fibrin glue. Another aspect of hydrogels that is a determinant of establishing a stable integration is whether the hydrogel dissipates the applied load efficiently or the forces concentrate on a region leading to crack propagation. With that in mind, more recently Karami et al. developed a DN hydrogel that, in addition to the high toughness due to chemical cross-linking of PEGDA and incorporating fiber reinforcement, which is critical for retarding crack initiation, a wide-range of physical interactions, including ionic and hydrogen bonds play two crucial roles in improving crack propagation [200]. First, because of their reversible nature, they considerably increase hysteresis manifesting energy dissipation through sacrificial bonds under loading, while unloading leads to elevated interfacial strength. Second, these physical interactions provide available sites in the hydrogel to interact with the tissue underneath (Fig. 9B).

2.6. Summary

The mechanical properties of hydrogels, such as stiffness and elasticity, must closely align with native cartilage to effectively distribute load and integrate with surrounding tissues. Balancing stiffness, toughness, and elasticity is challenging because improving one often compromises another. For example, increasing stiffness can reduce water retention, which is vital for cell viability. Innovative approaches, such as multi-network hydrogels or dynamic covalent bonds, are needed to create hydrogels that can endure the complex mechanical environment of joints while supporting tissue regeneration. Additionally, these materials must be compatible with cell encapsulation and non-cytotoxic. Biodegradability is also critical; the hydrogel must degrade at a rate that allows for structural support while gradually being replaced by a new ECM. Porosity and interconnectivity are key for cellular infiltration, nutrient diffusion, and waste removal, which are essential for functional tissue formation. Advanced hydrogels incorporate strategies like enzymatically degradable segments and microcavity structures to enhance matrix distribution and chondrocyte proliferation. However, integration with host tissue remains a major challenge, particularly due to the dense ECM of cartilage and the demanding mechanical environment of joints. Research is focused on improving integration through bioadhesives, modifying hydrogels with cell-attracting components, and optimizing pore architecture to support cell migration and ECM deposition. Overcoming these challenges is crucial to unlocking the full potential of hydrogels in cartilage repair and ensuring their successful clinical application.

3. Design of stratified-structural hydrogels

The zonal organization is crucial for the structure and function of cartilage, yet most current tissue engineering approaches regenerate cartilage with uniform properties. Stratified-structural hydrogels offer an innovative solution by mimicking the complex, layered structure of natural cartilage. These hydrogels can be designed as layered scaffolds (with two or three layers) or gradient scaffolds, each tailored to replicate specific areas of cartilage. In this section, we delve deeper into these types of hydrogels.

AC is segmented into four zones, each characterized by distinct levels of collagen fibers, PG content, and variations in chondrocyte count and shape (Fig. 10). The superficial (top) zone is characterized by the densest cell population with flattened morphology. The ECM mainly consists of Col II, while the concentration of GAGs and PGs is the lowest compared to the three other layers except PG4, which has a high concentration [202–204]. Moreover, this zone has a high expression of Sox9 and superficial zone protein (SZP) [205]. By shifting from a superficial to a middle zone, not only do cells gradually turn into larger chondrocytes, but they also alter from a flattened to a more spherical shape, and cell density decreases substantially [90,206]. There are also some alterations in biochemical composition. As the sulfated GAGs and PG start to increase, the Col II, PG4, and water amounts start to decrease [207]. Lastly, calcified (bottom) cartilage has less active chondrocytes with hypertrophic cartilage markers such as Col X and alkaline phosphatase (ALP). Chondrocytes, distributed across these zones, are each nestled within a dense PCM [206,207].

It is worth mentioning that this stratified structure gives particular properties and functionalities to each layer. The superficial zone has the highest tensile strength according to high Col content [208]. Additionally, PG4 and SZP play an important role in joint lubrication which is the main characteristic of this zone [205]. Increasing GAG content with depth causes an augmentation of the compressive modulus in the deep zone [209]. Finally, the presence of Col X and ALP makes it possible for the transition zone to anchor to the underlying subchondral bone [210]. Conventional tissue engineering methods for reproducing cartilage did not consider this zonal architecture and as a result, the fabricated neo-cartilage may lack full functionality with respect to load distribution, lubrication, and integration with natural tissue [211]. To imitate the stratified structure of cartilage, Klein et al. used subpopulations of chondrocytes from different cartilage zones, knowing that isolated chondrocytes from each layer show distinct biosynthetic activities. Although they attempted to construct a layer resembling the superficial zone with lower compressive properties and a high concentration of SZP [212], it should be noted that isolation of chondrocytes from different zones can be arduous [213,214].

Another strategy for recapitulating the zonal organizations is using biomaterials with varying biomechanical and biochemical properties to resemble the microenvironment of natural cartilage for embedded cells inside them [215]. These variable properties include gradients in stiffness [215], topography [216], diffusive biomolecules [217–219], composition [220], and pore size [221]. Besides, dissimilar cell density could be loaded in each layer corresponding to each layer's density in natural cartilage [217–219]. Furthermore, to imitate the microenvironment cells encounter in natural cartilage, attempts have been made to make gradient oxygen tension in fabricated constructs and many studies have been done to evaluate the effect of applied mechanical forces on the zonal organization of final tissue during cell culture [214,222]. Hydrogel is a cell-friendly template that not only resembles natural cartilage in terms of the water ratio trapped in its structure but also can be tuned for establishing one or more of the gradients.

The average modulus of superficial, middle and calcified zones are 0.08, 2.1, and 320 MPa, respectively [217]. The PEG-based hydrogels are the most practical platform that has been used for creating gradient stiffness. Modifying this synthetic hydrogel with a natural polymer such as HA and CS benefits the scaffold by better-directing cells to ones that can synthesize the specific-layer ECM [220,223]. Moreover, RGD is incorporated into the PEG-based hydrogel to enhance the ability of chondrocytes to detect changes in mechanical properties [224]. While increasing matrix stiffness led to higher expression of the genes encoding Agc, Col II was also expressed more in the stiffer zone. The most recent trend contrasts with that of natural cartilage. Results from this study demonstrate that a sole gradient may not necessarily result in the stratified organization like natural cartilage and the authors claim

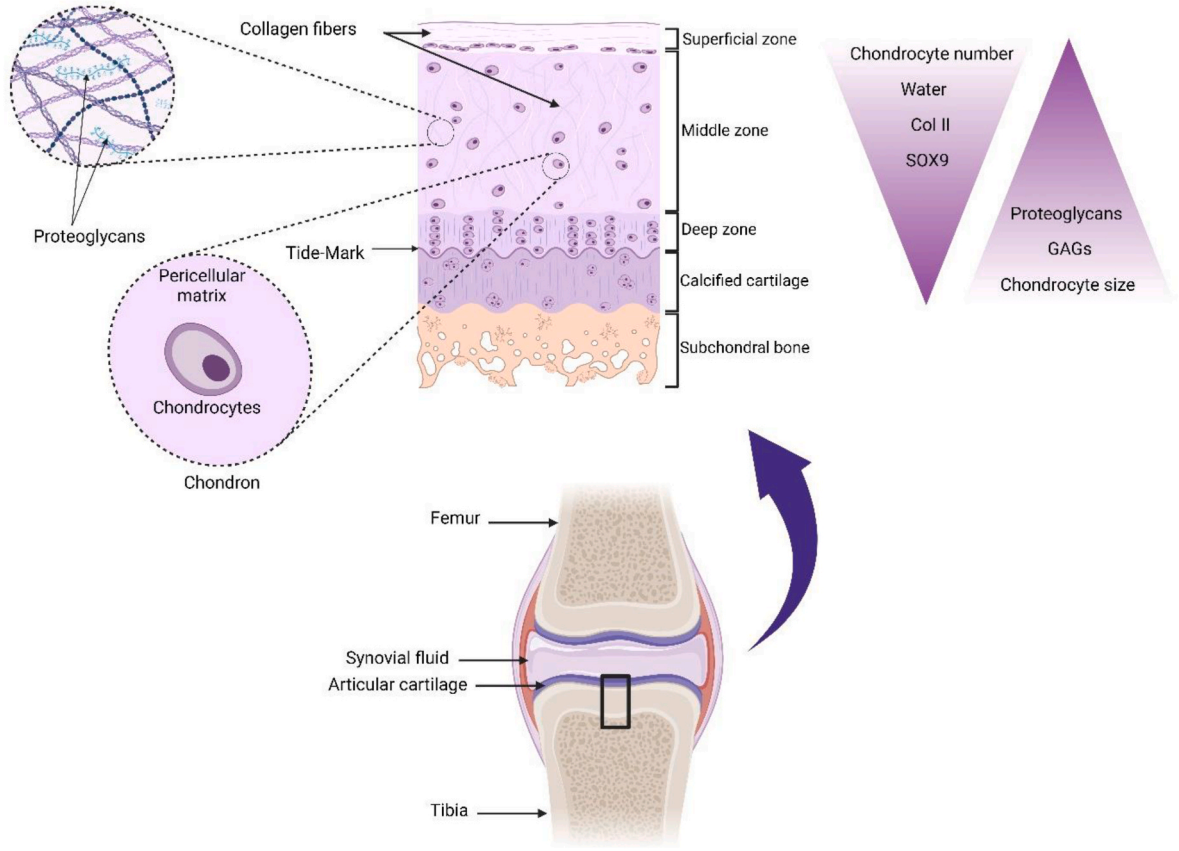

Fig. 10. Schematic illustration of the anatomical structure of AC with four histological zones. The extracellular matrix (ECM) of AC is a complex assembly of Col II and PGs. This intricate composition influences the alignment of Col fibers, cellular density, and cytomorphology, fostering a cartilage structure with a hierarchical organization. Progressing from the superficial to the calcified zones, there's a discernible shift in cell density and phenotype, accompanied by changes in the tissue's architecture and chemical makeup.

additional cues are required to achieve that aim [215].

The results from previous studies should be treated with caution since different cell types may respond differently to biomechanical and biomechanical cues [220]. Chondrocytes are more capable of degrading hydrogels during the embedded time and are more likely to proliferate in the stiffer zones [215]. Besides extrinsic cues, concerning intrinsic properties of cells could lead to better choices of encapsulated cells in each layer. Levato et al. have shown that articular cartilage-resident chondroprogenitor cells (APCs) offer greater benefits than MSCs and chondrocytes in terms of populating the template within the superficial zone. This type of cell not only has a differentiation capacity after extensive culture expansion [225] but also has a high expression of PG4 and the lowest expression of Col X [226]. To reach a neo-cartilage comparable to native cartilage regarding the amount of ECM, the appropriate cell density should be loaded. PRG4 expression increased when 50×10^6 cells ml^{-1} were seeded on the hydrogels rather than 20×10^6 cell ml^{-1} [214].

Not only does Col fibrils alignment play a pivotal role in the biomechanical properties of natural cartilage, but various sizes and orientations through the depth of adult AC also guide the cells in expressing more zone-specific ECM. One of the beneficial features of the decellularized matrix being used as a biomaterial is that it can preserve the nanostructure of initial tissue after removing cells [227]. Given that skeletal maturity establishes Col fibril alignment and increases its diameter, which supports cell proliferation, Luo et al. explored how this alignment impacts chondrogenesis by comparing Col and GAG produced by mature and immature decellularized explants repopulated by fat pad-derived stem cells [228]. They observed more proliferation (13-fold increase to 5-fold increase in DNA content) and a higher Col: GAG ratio (1.4 vs 0.4) in mature scaffolds.

The manipulation of cells' fate through micro and nanostructures is a well-known fact. In the utilization of hydrogel, there are two distinct approaches for fabricating biomimetic structures that closely resemble the ones encountered by cells in their natural environment. These approaches are composite hydrogels and micropatterned hydrogels. Whilst the primary aim of incorporating fibril into hydrogel is advancing its mechanical properties, it also mimics the composite structure of natural cartilage [229,230]. Mellati et al. used photolithography techniques to create 3D patterned microstructures to direct cell organization [231]. They observed that among 50, 100, and 150 μm wide micro constructs, the smaller channel width had the higher cell alignment, which resembles the geometry of chondrocytes in the superficial zone. The induced phenotype corresponds to the synthesis of zone-specific ECM [232].

The size of cell-seeded in hydrogels matters when biomimetic microenvironments such as hypoxia and mechanical load on respective tissue are applied to engineered tissue. Hydrogels infused with fibro chondrocyte-like stem cells (FPSCs) were employed, with variations in height (either 2 mm or 4 mm), and subjected to two distinct boundary conditions: confinement to half of their thickness and/or dynamic compression (DC). It was theorized that confining FPSC-laden hydrogels would serve to accentuate the oxygen tension gradient within the constructs. Specifically, this would result in higher oxygen levels at the top and lower oxygen levels towards the bottom of the hydrogels. This, in turn, was anticipated to promote an upswing in the synthesis of GAG and Col, particularly in the 2 mm high constructs. Moreover, when exposed solely to dynamic compression (DC), both GAG and Col accumulation displayed an upward trajectory within the unconfined 2 mm high

constructs. This was accompanied by a noteworthy elevation in the dynamic modulus of the constructs, rising from 0.96 MPa to 1.45 Mpa following the application of DC. Nonetheless, when considering the combined influence of confinement and DC, no synergistic advantages were discerned regarding the overall levels of matrix accumulation. Notably, the superficial region of these constructs exhibited a striking resemblance to native tissue. It exhibited weak staining for GAG, strong staining for Col II, and in the case of the 4 mm high tissues, a more pronounced staining intensity for PG4 [222]. The Jabbari group conducted several studies attempting to recapitulate the zonal organization of natural cartilage by utilizing a combination of variable stiffness, cell density, GFs and fiber orientation so that each factor encourages the particular layer to resemble more than the respective layer in natural cartilage [217,218].

Multi-layered hydrogels based on the photo-polymerization technique is the most common technique that has been used for creating hierarchical hydrogels. In this technique, each material composition is placed on a mold sequentially and combined with a photo initiator then exposed to the UV light for a specific period. The next layer is then added on top of the previous layer, and the same approach is repeated until a single unit is acquired [220,233]. One potential limitation of the constructs made by this technique is lack of integrity, which leads to delamination under mechanical stress. To overcome this limitation, Zhu et al. set up a process in which two different concentrations of cell-containing precursor solutions combined in the mixing chamber were entered into a mold and subsequently exposed to the UV light in a way that the stiffness of the final construct ranged from 2 kPa to 60 kPa [215]. They also assessed the viability and cell distribution among their

Table 5
A summary of cartilage scaffolds with different stratified strategies and regional characteristics of the cartilage region.

Stratified-structural type	Material	Hydrogel type	Outcome	References
Cell properties and phenotypes	Col II	3D-bioprinted (trilayered)	• Zonal distribution of ECM	[234]
	F-CarMa, and a-CarMa	Injectable	• Increased differentiation to the superficial zone phenotype in high cell density in f-CarMa by sequential addition of IGF-1 and IHH and to the calcified zone in a-CarMa in low cell density	[235]
	8-arm PEG-norbornene, PEG-dithiol, and CS-MA	Gradient	• Faster cartilage deposition occurs for MSCs in a soft matrix (Young's modulus <5 kPa) and for chondrocytes in a stiffer matrix (Young's modulus >20 kPa)	[236]
Zone-specific GFs	AC of bovine legs	Multi-layered photopolymerizing gels	• Differentiation of hMSCs into the middle zone phenotype due to the addition of IGF-1 to cartilage medium/TGF-β1 • Differentiation of hMSCs into calcified zone phenotype due to the addition of IHH to cartilage medium/TGF-β1	[237]
	F-CarMa, and a-CarMa	Injectable	• Differentiation to the middle zone phenotype by adding IGF-1 to chondrogenic medium/TGF-β1 and BMP-7 • Differentiation to the calcified zone phenotype by adding IHH to chondrogenic medium/TGF-β1 and BMP-7 and IGF-1	[235]
Porosity	PEGSA	3D-printing (bilayered hydrogel)	• Swelling altered the pore size of the small-pore layer and let BMSCs infiltrate at an early stage • The large-pore layer enhances BMSCs' chondrogenic differentiation by providing a soft, viscoelastic, and biocompatible environment	[156]
Matrix composition	CS, HA, and PEGDA	Multi-layered hydrogel	• Directing hBMSCs to distinct and zone-specific cell phenotypes by layer-specific composition	[220]
Fibril orientation	HA, PLA	Zonal scaffold (tri-layer)	• Aligned nanofiber mesh in the superficial zone caused cell elongation, lower GAG and Col II, and higher proliferation and Col I than the middle zone cells • Random nanofiber mesh in the middle zone grouped cells and raised Col II • The deep zone scaffold had the highest GAG, lowest proliferation and Col I of all zones	[238]
	PEG, cellulose nanofiber	Composite hydrogel (trilayered)	• The morphologies and Col II content distribution of cells cultured on each zone of the hydrogel composite resemble those of natural articular cartilage • The deep zone frame's nutrition and cell transport behaviors facilitated the CR process	[239]
	Ch, Gel	Multizonal scaffold (- Top zone with horizontal pores - Deep zone with vertically aligned pores - The transition with zone randomly oriented pores)	• Under cyclic compression, these scaffolds showed high viscoelasticity and their pore orientation affected how much energy they dissipated	[240]
Mechanical properties	8-arm PEG-norbornene, PEG-dithiol, CS-MA	Gradient Hydrogel	• Faster cartilage deposition for MSCs in a soft matrix with a Young's modulus less than 5 kPa and for chondrocyte cells in a stiffer matrix (Young's modulus >20 kPa)	[236]
	Ch, Gel	Multizonal scaffold	• The lowest moduli for the horizontal layer • The highest moduli for the vertical layer • The moderate moduli for the random layer	[240]
	Col, Ch–PCL	Low-temperature deposition processing technique (four-layered scaffold)	• 10 % increase in compressive modulus and stress from the upper layer to the lower layer • Creating a swelling index gradient	[241]
	PEG, cellulose nanofiber	Composite hydrogel (trilayered)	• The compressive moduli of the superficial zone 298 kPa • The compressive moduli of the middle zone 182 kPa • The compressive moduli of the deep zone 9.8 MPa	[239]
	Agarose	bilayered construct	• More mechanical strength in the bottom layer • More Col production in the top layer	[242]

Abbreviations: F-CarMa: Methacrylated fetal cartilage, a-CarMa: Methacrylated adult cartilage, CS-MA: chondroitin sulfate methacrylate, PEGSA: acrylated-poly (glycerol sebacate)-co-PEG.

constructs by live-dead staining analysis and observed high viability and homogenous cell distribution throughout the hydrogel. In 3D-printing, a platform can be used to create area-dependent hydrogels by applying multiple gradients simultaneously [226,233]. Table 5 summarizes the cartilage scaffolds with stratified structure and regional characteristics of the cartilage region, including cellular properties and phenotypes, region-specific GFs, matrix compositions and porosity, Col fiber orientation, and mechanical properties.

Stratified-structural hydrogel scaffolds can be expanded with a bone layer to create osteochondral scaffolds for repairing osteochondral defects. These scaffolds need to support both cartilage and subchondral bone formation. Lin et al. used a solvent-free urethane cross-linking method to create PEGylated poly (glycerol subcategory) (PEGS) scaffolds with controllable porosity and cross-linking degrees [243]. The low cross-linked PEGS-12 h variant promoted chondrogenic differentiation and cartilage matrix secretion. Additionally, PEGS-12 h was combined with osteoinductive bioactive mesoporous glass (MBG) to form PEGS/MBG bilayered scaffolds. Gao et al. used 3D-printing to create bilayered scaffolds with GelMA for the cartilage layer and a GelMA-HAp mix for the subchondral layer, which showed potential for *in vivo* CR [244]. Gong et al. developed a double-layer 3D-printed scaffold with interleukin-4 in the upper GelMA layer and a porous PCL-hydroxyapatite lower layer [245]. In another study conducted by Rajzer et al., a synergistic approach involving two scaffolding techniques—3D-printing via fused deposition modeling (FDM) and electrospinning—was adopted to create a layered scaffold. The resulting hybrid layered scaffolds consisted of a top layer composed of Gel nanofibers and a 3D-printed porous poly (L-lactic acid) (PLLA) material [220]. Pasty calcium phosphate cement (CPC) and a bioink based on Alg-methylcellulose (AlgMC) were also applied in a monophasic and combinatory design to recreate osteochondral tissue layers. The chondrogenic fate of cells in co-fabricated and biphasic CPC-AlgMC patterns was also evaluated. Most cells survived and redifferentiated in the AlgMC hydrogel, producing ECM components after 3 weeks [246].

Delamination of the two layers often leads to tissue disjunction and limited clinical applications. To address this, Li et al. developed a PCL-based scaffold with a strong interface [247]. The upper layer was an ECM-coated 3D-printed composite scaffold (E-co-E/PCL), while the bottom layer was a MgO@PDA incorporated 3D-printed composite scaffold (MD/PCL). This bilayered scaffold exhibited suitable physicochemical and mechanical properties. Xing et al. reported an integrated bilayered hydrogel with a strong interface (40 kPa), facilitating calcium ion diffusion into the secondary cross-linking of the bilayered hydrogel [248]. The cartilage layer consisted of gellan gum (GG) and Alg, while the subchondral layer comprised GG and hydroxyapatite. This structure demonstrated high cell compatibility and could be seeded with MSCs that expressed various proteins for cartilage and bone formation. In a rabbit model with a critical osteochondral defect, the calcium-rich hydrogel served as a calcium source, induced neovascularization by week 4, and repaired the defect by week 8. Multiphasic scaffolds also play a role in inducing angiogenesis in the subchondral bone. Wang et al. employed a triphasic GelMA hydrogel scaffold with a CS and hydroxyapatite gradient, along with covalent bonding, to repair osteochondral defects [249]. This scaffold induced chondrogenesis of BMSCs, angiogenesis, and osteogenesis and had gradient mechanical strength. Evaluated in a rabbit model, it accelerated osteochondral regeneration, suggesting a novel treatment strategy.

The emergence of stratified-structural hydrogels offers a promising alternative by mimicking the layered architecture of cartilage, which is crucial for load distribution, lubrication, and integration with surrounding tissues. These hydrogels can be designed with distinct layers or gradients that reflect the varying mechanical and biochemical properties across the cartilage zones, such as differences in Col content, GAG levels, and cell morphology. Recent advancements in hydrogel design incorporate gradients in stiffness, topography, and biochemical cues, aiming to recreate the natural environment that cells encounter within the cartilage matrix. Techniques such as 3D printing have enabled the creation of multi-layered scaffolds that can better mimic the mechanical and functional properties of native cartilage. However, challenges remain in achieving strong layer integration and optimizing cell and matrix distribution for effective tissue regeneration.

4. The influence of environmental stimuli on hydrogel design

Hydrogels have been recognized as potential scaffolds for articular CR. Environmental stimuli, both biochemical and biophysical, significantly influence their performance and function [250]. Designing hydrogels requires careful consideration of these external factors to optimize their characteristics and functionality. This section explores how external stimuli impact hydrogel design for articular CR. By understanding this relationship, we aim to develop better strategies for cartilage repair, ultimately improving clinical outcomes and quality of life for individuals with cartilage damage or degeneration.

4.1. Biochemical stimuli

Biochemical stimuli play crucial roles in cartilage tissue regeneration by regulating cellular activities such as proliferation, differentiation and matrix synthesis. In the context of hydrogel design, incorporating these biochemical agents within the hydrogel matrix can enhance the biological response and promote CR. Different types of biochemical stimuli that are used include GFs, cytokines, small molecules and molecules derived from the extracellular ECM [251]. One of the main challenges in cartilage tissue engineering is delivering biochemical stimuli to cells within the hydrogel matrix in a controlled spatiotemporal manner [252]. Integrating these stimuli into hydrogel matrices and developing strategies for their controlled release and spatial distribution is crucial for enhancing the efficacy and safety of cartilage tissue engineering. Several methods have been developed to incorporate biochemical stimuli into hydrogel matrices, including.

- Physical entrapment: Biochemical agents are physically incorporated into the hydrogel network, typically releasing at a slow and steady rate, though sometimes exhibiting an initial rapid burst [252,253].
- Covalent attachment: Functional groups of hydrogel precursors bind biochemical agents covalently, helping to keep them in place and/or release them in a controlled manner [254]. Amphiphilic polyurethanes with amine groups were created to form amide bonds with Kartogenin's (KGN) carboxyl group. The resulting KGN-conjugated polyurethane NPs were uniform spheres, averaging 25 nm in size, and demonstrated controlled KGN release *in vitro* [255].
- Affinity binding or encapsulation: Biochemical stimuli can be encapsulated within micro- or nano-carriers or loaded into electrospun fibers, offering more control and longer duration of release. These carriers can be designed with various sizes, compositions, biodegradability, surface shapes, and functions to adjust release rates for different applications [256,257].

These methods protect biochemical stimuli from degradation and clearance, allowing for sustained or stimuli-responsive release based on factors like the degradation rate of the hydrogel or carrier, the diffusion coefficient of the stimuli, or environmental cues such as pH, temperature, enzymes, or light [252,253]. Additionally, the spatial distribution of biochemical stimuli within hydrogel matrices can be achieved using techniques like gradient generators, microfluidic devices, layer-by-layer assembly, or 3D-printing. These techniques create spatially varying concentrations or patterns of biochemical stimuli within hydrogel matrices, mimicking the natural gradient of signaling molecules in tissues and inducing directional cell migration, alignment, or differentiation [253,258].

4.1.1. Growth factors

Growth factors (GFs) are crucial in regulating cell behavior, proliferation, and differentiation. Key GFs used in CR studies include TGF-β, bone morphogenetic proteins (BMPs), IGF-1, and PDGF. Clinically, GFs can be administered through direct injection into the affected area or systemically as recombinant variants. However, their use is limited by factors such as large molecular size, slow tissue penetration, short half-life, and potential systemic toxicity [252,259]. To address these challenges, Guo et al. developed a dynamic hydrogel (Pep-GelSH) using thiol-modified Gel and thiol-capped TGF-β1-affinity peptide through Au-S coordination. This hydrogel showed superior attachment to host tissues and facilitated rapid penetration of host cells. It significantly increased the local concentration of endogenous TGF-β1, effectively attracting stem cells (Fig. 11A) [260]. Research indicates that a combination of GFs can effectively attract cells. Liebesny et al. found that neither PDGF nor TGF-β1 alone could induce MSC migration, but their combination successfully did [261]. Another study demonstrated that combining microgels with GFs and enzymatic cross-linking formed heterogeneous granular hydrogels embedded with GFs. Sulfated microislands loaded with GFs directed cell migration and significantly promoted chondrogenesis [262].

4.1.2. Small molecules

Small molecules can significantly influence cellular processes such as proliferation, differentiation, and matrix synthesis. They offer advantages over GFs in terms of stability and cost, making them attractive for CR [266]. KGN is a notable small biomolecule that specifically promotes the chondrogenesis of MSCs. Other small molecules used in CR include melatonin (MEL), glucosamine (GlcN), and resveratrol (RSV) [267]. Shi et al. developed a hydrogel incorporating KGN-loaded PLGA microparticles using photo-crosslinked HA for CR [268]. Complementing this, Wang et al. created an injectable HA hydrogel with RSV-loaded PLGA microspheres designed for sustained release over one month (Fig. 11B) [264]. Liu et al. found that combining small molecules with TGF-β enhances chondrogenesis. They studied the combined effect of KGN in liposomes (KGN@Lipo) and Cytomodulin-10 (CM-10), a TGF-β-like peptide, in GelMA hydrogel (Fig. 11C). Their results showed that CM-10 on GelMA hydrogels was as effective as TGF-β1 in promoting chondrogenesis, an effect further amplified with KGN. Additionally, CM-10 and KGN synergistically boosted chondrogenesis in bone marrow stem cells by increasing RUNX1 and SOX9 expression at the mRNA and protein levels [263].

4.1.3. ECM-derived molecules

Native cartilage ECM contains various biochemical agents that can

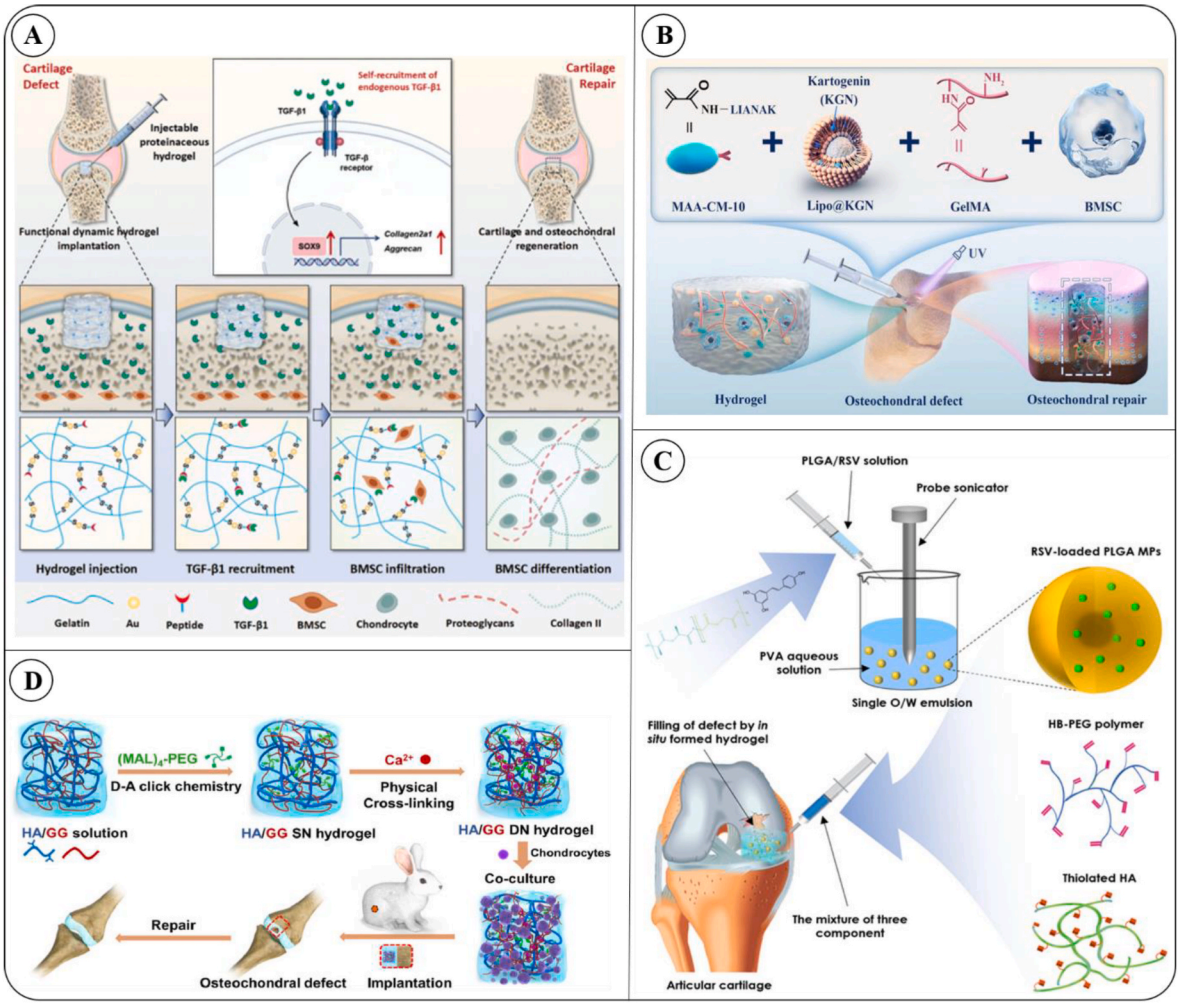

Fig. 11. Schematic illustration of A) The function of the TGF-β1-affinity peptide modified dynamic proteinaceous hydrogel (Pep-GelSH) in in-situ CR. Reproduced with permission [260]. Copyright 2024, published by John Wiley and Sons. B) The fabrication of the composite hydrogel loaded with BMSCs, CM-10, KGN@Lipo, and GelMA for the repair of the osteochondral defect. Reproduced with permission [263]. Copyright 2023, Elsevier. C) Integrated drug delivery system for CR. Reproduced under the terms of the CC BY 4.0 license [264]. Copyright 2021, published by John Wiley and Sons. D) The preparation of HA/GG DN hydrogel as a biomimetic ECM for rabbit cartilage defect regeneration. Reproduced with permission [265]. Copyright 2022, Elsevier.

be utilized to enhance CR [227]. ECM-derived molecules such as Col, CS, HA, and cartilage acellular matrix (CAM) fragments can mimic the natural microenvironment and promote chondrogenesis [269]. Barthold et al. engineered an innovative ink composed of particulate extracellular matrix (pECM) and functionalized hyaluronan [270]. This bio-ink can be smoothly extruded and has been shown to enhance the survival rate of embedded cells by 10 %. Additionally, research has unveiled a DN hydrogel crafted from HA/GG. This hydrogel stands out for its high compressive strength, toughness, stiffness, and good self-recovery properties. It also fosters the proliferation and preservation of cartilage cells and the formation of new ECM (Fig. 11D) [265].

Selected published works are summarized in Table 6 to exemplify the exploitation of these biochemical stimuli in designing hydrogels for articular CR.

4.2. Biophysical stimuli

Environmental biophysical stimuli are crucial in cartilage tissue engineering alongside cells, scaffolds, and GFs/cytokines [292]. Mechanical, electrical, and electromagnetic stimuli significantly influence cartilage behavior, affecting cell migration, differentiation, morphology, proliferation, and gene expression [293]. These stimuli play a vital role in regulating cartilage under both normal and pathological conditions [294,295].

Research indicates that the application of pulsed electromagnetic fields as a form of biophysical stimulation enhances the recovery of patients with cartilage damage in the knee, showing positive effects in both short and long-term outcomes. Consequently, biophysical stimulation emerges as an effective tool for improving the clinical results of regenerative medicine [296,297]. Biophysical stimuli can be used in particular in matrix-induced autologous chondrocyte implantation (MACI). In MACI, autologous chondrocytes are harvested from the patient and cultivated on a three-dimensional biocompatible scaffold [298]. Once a sufficient number of cells has been obtained, they are implanted into the damaged cartilage area in a second surgical procedure. During cultivation, the proliferation and differentiation of the cells can be supported by various biophysical stimuli using a bioreactor. Furthermore, the significance of physical stimuli extends to the development of hydrogels that mimic the intricate microenvironment of natural cartilage. Hydrogels designed with this understanding have the potential to significantly enhance tissue regeneration.

4.2.1. Mechanical stimuli

Mechanical stimuli involve applying forces, movements, or deformations to a material or structure. In hydrogel design, this means subjecting the hydrogel to physical forces such as compression, tension, or shear (Fig. 12A) [295]. By applying these mechanical stimuli to hydrogels containing cartilage cells, researchers can enhance the formation and quality of new cartilage tissue. Cells within the hydrogel respond to mechanical cues, leading to changes in cell morphology, alignment, and the production of extracellular matrix components [299]. Integrating mechanical stimuli into hydrogel design is crucial for creating biomimetic materials that closely mimic the mechanical environment of native tissues.

Mechanical stimuli can influence the cell-encapsulated hydrogels in various ways, such as.

- Improving the mechanical properties of the hydrogels, such as stiffness, strength, and elasticity [300];
- Increasing the adhesion of the hydrogels to the native cartilage tissue, which is important for integration and stability;
- Stimulating the production of cartilage-specific molecules, such as Col and PGs, by the cells within the hydrogels;
- Regulating the gene expression and signaling pathways of the cells within the hydrogels can affect their differentiation, proliferation, and survival [250,300,301].

Bioreactors are specialized systems designed to apply controlled mechanical forces to cells and tissues, playing a crucial role in tissue engineering [302]. They can control factors such as strain, frequency, duration, and loading modes, simulating the physiological loading conditions of native cartilage [303]. By using bioreactors to apply mechanical forces to hydrogel scaffolds, researchers can influence cell behavior, enhance extracellular matrix production, and direct tissue development toward more natural cartilage phenotypes [302,304]. They come in various configurations, including stirred, perfusion, rotating wall vessel, stretch, compression, hydrostatic pressure, and combined systems (Fig. 12B) [208]. These systems facilitate nutrient and waste exchange and control oxygen tension, ensuring optimal cell viability and metabolic activity within hydrogel constructs [304,305]. Bioreactors are essential in cartilage tissue engineering for their ability to apply noncontact forces, such as magnetic or electric fields, and control environmental conditions like hydrogel type, composition, ion concentrations, pH levels, and temperatures. They offer two main advantages: One is to mimic the physical and biomechanical conditions for cartilage growth and development. The other is to measure tissue status and chondrocyte behavior using digital image processing technology, allowing for the refinement of *in vitro* cartilage development by evaluating different culture conditions [306].

Biomechanical loading has been shown to preserve articular cartilage in several studies. Tran et al. created tissue-engineered cartilage from porcine chondrocytes without a scaffold by centrifuging a high-density chondrocyte resuspension on an agarose layer. They improved the biomechanical and biochemical properties of their constructs by using a bioreactor with biomechanical stimulation, producing large tissue-engineered cartilage [307]. Bian et al. used agarose hydrogel with adult canine chondrocytes and applied dynamic loading to test its effectiveness. They used bioreactors to apply unconfined axial compressive deformational loading and sliding contact loading methods for 3 h per day [308].

The scaffold can withstand various frequencies and loads, which should be selected based on the specific target tissues. For cartilage tissue, the load and frequency of loading should mimic human walking. Natenstedt et al. found that the optimal condition for cartilage tissue was 5–10 MPa with a frequency of 1 Hz for a week or more [309]. Kowsari-Esfahan et al. used a microfluidic device for unidirectional compressive stimulation of cells and demonstrated that 10 % strain (among 0, 5, 10, 15 and 20 %) was the best to induce chondrogenesis in ADSCs encapsulated in Alg hydrogel [310]. On the other hand, AC has different zones with different properties. The biomechanical load condition in the cartilage differs in the zones, with the highest strain in the superficial zone and the lowest in the deep zone. Chondrocytes on the surface experience both compressive and shear strains, while stimulation in the deeper layers is mainly compressive. This biomechanical load results in a different elastic modulus; it is lower in the superficial zone than in the deep zone [301,311]. The integration of bioreactors with hydrogel systems for cartilage tissue engineering allows for the application of physiologically relevant mechanical forces, which are known to modulate cellular behavior, stimulate matrix synthesis and guide tissue formation. Furthermore, the use of bioreactors enables the customization of mechanical stimulation parameters, including magnitude, frequency and duration, tailored to specific tissue engineering goals and experimental requirements [302].

Meinert et al. developed a system for controlled uniaxial or biaxial mechanical stimulation to grow cartilage tissues. Using GelMA and methacrylated HA hydrogels, they showed that mechanical stimulation increased the expression of hyaline cartilage-specific genes and enhanced matrix production [312]. Stokovska et al. tested a bioreactor to evaluate cell-hydrogel interactions under *in vivo*-like conditions. They used Alg hydrogels in disc and microbead shapes, applying various mechanical stimulations. They found that hydrogel mechanical properties depended on Alg concentration, composition, and shape. Bovine calf cartilage cells cultured on microbeads under dynamic compression

Table 6
A summary of biochemical stimuli in hydrogel design for cartilage applications.

Biochemical stimuli	Name	Incorporation methods	Release profile	Performance	References
Growth factor	TGF-β	Physical	Sustainable release without the initial burst (after 120 h, about 30 % of the drug remained inside)	• Enhance cartilage production in rat knee joint model	[271]
		Physical	–	• Stimulate the proliferation and chondrogenic differentiation of BMSCs *in vitro* • Enhance synthesis of GAGs • Full-thickness cartilage defect repair by hyaline cartilage in rat model	[272]
		Covalent attachment	Sustainable release within 21 days without initial burst (Only a 7–8% overall release was detectable after 21 days.)	• Enhance synthesis of GAGs and Col II	[254]
		Encapsulation within nanocarriers	Sustainable release within 21 days without the initial burst	• Increase expression of Sox9, Col II and Agc genes • High adhesion, proliferation, and differentiation in hDPSCs	[273]
	BMPs	loading into fibrous scaffolds in the form of NPs	Initial burst release within 12 h and continuous release during 2 weeks	• Increase in cartilaginous micro-tissue formation and increase in sulfated PGs • High induction of PG and Col II formation of thick hyaline cartilage in rabbit osteochondral defect	[274]
		Transfected BMSCs with lentivirus loaded with BMP's gene	Sustainable release of more than 28 days	• Enhance adhesion and proliferation of BMSCs • Reconstruction of defects during 12 weeks after implantation in knee joints of beagle dog model	[275]
		Physical	Sustainable release	• Enhance cartilage production in rat knee joint model	[276]
	IGF-1	Encapsulation within the bilayered microsphere	Sustainable release within 20 days without the initial burst	• The ability to inhibit the tube formation of HUVECs • Decrease in osteogenic and vasculogenic gene expression and an increase in chondrogenic gene expression	[277]
		Physical	Sustainable release within 3 weeks	• Increase glycosaminoglycan deposition and expression of cartilage-related genes in ADSCs • Reconstruction of defects during 12 weeks after implantation in a rabbit full-thickness osteochondral defect model	[278]
	PDGF	Physical	70 % release within two weeks	• Enhance adhesion and proliferation of ADSCs • Increase expression of Sox9, Col II and Agc genes	[279]
		Physical	Initial burst release during a week (20 %) followed by sustainable over 4 weeks (30 %)	• Enhance adhesion and proliferation of BMSCs • Increase expression of Sox9, Col II and Agc genes • Enhance cartilage production in rat knee joint model	[141]
		Physical	35 % release within a week	• Stimulate progressive migration of MSC • Creating an amenable microenvironment for cartilage production after subcutaneous implantation in athymic mice	[280]
Small molecules	KGN	Covalent attachment	41 % release within a week	• Increase expression of Sox9, Col II and Agc genes • Increase the level of defect regeneration after 12 weeks in the rabbit osteochondral defect model	[256]
		Encapsulation within the microsphere	Initial burst release during 3 days and continuous during 28 days	• Enhance cell viability, proliferation, and adhesion of SMSCs • Enhance synthesis of GAGs and Col II	[281]
		Covalent attachment	Sustainable release during 192 h	• Increased expression of chondrogenic genes and protein markers in DPSCs	[282]
	MEL	Encapsulation within the NPs	Initial burst release during 3 days (60 %) followed by sustainable during 15 days (90 %)	• Enhance chondrocyte proliferation, differentiation, and preservation of its phenotype • Increase expression of chondrocyte-specific genes	[283]
		Encapsulation within microparticle	Initial burst release during 10 days (25 %) followed by sustainable of the remaining during 90 days	• Decrease in expression of Col I gene and increase in expression of Sox9, Col II, and Agc genes • Enhance synthesis of GAGs • Increase defect regeneration in the rabbit knee joint model	[284]
	GlcN	Covalent attachment	Significant increase within the first 2 h, reaching the maximum value after 4 h	• Good biocompatibility • Increase defect regeneration in the rabbit knee joint model	[285]
		Physical	Sustainable release	• Significant decrease of swelling and inflammatory factors and increased defect regeneration in the rabbit knee joint model	[286]
	RSV	Encapsulation within microparticle	Sustainable release during 32 days (78 %)	• Increase expression of Sox9, Col II, and Agc genes	[264]
		Encapsulation within microparticle	Reaching a plateau in the first week (3 %)	• Increase expression of Col II and Agc genes	[287]
ECM-derived molecules	Col	Physical combination	–	• Enhance synthesis of GAGs • Increase defect regeneration in the rabbit femur model	[288]
	CS	Covalent attachment	–	• Excellent adhesion and/or bio-integration of the hydrogel/BMSCs composite	[289]
	HA	Covalent attachment	–	• Increase cell viability and proliferation • Increase expression of Sox9, Col II, and Agc genes	[290]

(*continued on next page*)

Table 6 (*continued*)

Biochemical stimuli	Name	Incorporation methods	Release profile	Performance	References
	CAM	Physical loading in the form of powder	–	• Increase cell viability • Increase chondrogenic-like differentiation of hASCs and enhance the synthesis of GAGs • Increase expression of Sox9, Col II, and Agc genes	[291]

Abbreviations: HUVECs: human umbilical vein endothelial cells, SMSCs: synovium-derived mesenchymal stem cells, hASCs: human adipose-derived stem cells.

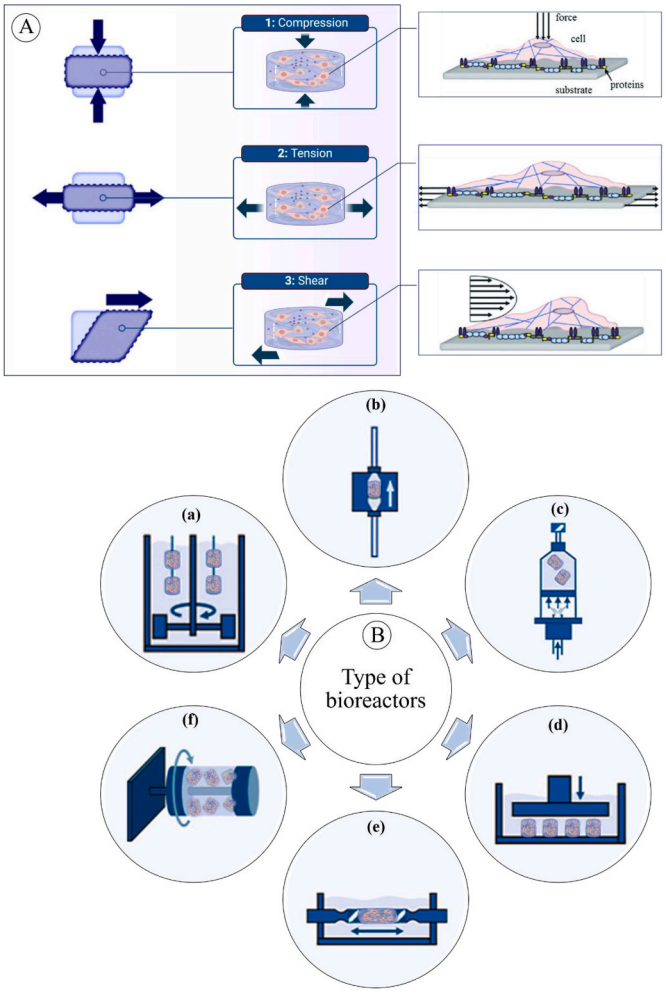

Fig. 12. Schematic illustration of different A) mechanical stimuli on cell-encapsulated hydrogels. Cells experience compressive, tensile, and shear stresses due to external mechanical stimuli. Compressive and tensile forces act perpendicularly to the cell membrane, whereas shear stress causes a change in the angle between the cell's opposite sides; B) designs of bioreactors mimicking the biomechanical characteristics of articular cartilage. (a) A stirred bioreactor system, (b) a perfusion bioreactor system, (c) a hydrostatic pressure bioreactor, (d) a compression bioreactor system, (e) a stretch bioreactor system, and (f) a rotating wall vessel bioreactor. Reproduced under the terms of the CC BY-NC-ND 4.0 license [208]. Copyright 2021, The Authors, published by Elsevier.

grew and compacted the microbeads, slightly increasing hydrogel mechanical strength [313]. Daly et al. explored dynamic bioreactor culture under different oxygen levels for developing large cartilage tissues using hydrogels with MSCs. They found that at 20 % O_2, dynamic culture inhibited chondrogenesis in all hydrogel sizes. However, at 3 % O_2, dynamic culture enhanced cartilage matrix component distribution and amount in larger hydrogels compared to static culture [314]. Zhou et al. created tissue-engineered cartilage *in vitro* using a hybrid hydrogel and human ADSCs (hADSCs) cultured in both dynamic and static settings [315]. They used Computational Fluid Dynamics (CFD) to verify mathematical simulations of glucose and TGF-β2 mass transfer in a rotating wall vessel bioreactor (RWVB) and static culture conditions during the early stages. The RWVB was then used to create 3D hydrogel cell-cartilaginous structures dynamically. The findings showed that RWVB achieved mass transfer equilibrium faster than static culture.

4.2.2. *Electrical stimuli*

Electrical stimuli in hydrogel design for cartilage repair involve applying electric fields or currents to influence the hydrogel and the cells within it [316]. This is important for cartilage because AC has inherent electrical properties, coming from the free electrolytes (K^+, Ca^{2+}, Na^+) flowing through the carboxyl and sulfate groups with fixed negative charges on the GAGs in the PG side chains. The uneven distribution of fixed charges in the tissue also generates diffusion potentials, while fluid flow across the charged tissue causes a streaming potential. Chondrocytes in the local area respond to these electrical signals, transforming them into intracellular signaling. This leads to a signal transduction cascade that produces Sox9, a transcription factor that stimulates the synthesis of typical cartilage ECM components such as Agc and Col II [293,316]. Various types of electrical stimuli, such as direct current, alternating current, capacitive coupling, or electromagnetic fields, can be applied in different intensities, durations, and frequencies. These stimuli can affect the cell-encapsulated hydrogels in various ways, such as.

- Modulating the electrical conductivity and charge of the hydrogels, which can affect cell adhesion, migration, and alignment [316];
- Activating the ion channels and receptors on the cell membrane, which can affect cell signaling, gene expression, and metabolism [317];
- Inducing the electrophoresis and electro-osmosis of the ions and water within the hydrogels can affect nutrient transport, pH balance, and swelling behavior [316].

Conductive materials, such as conductive polymers [318,319], metal NPs [320], graphene, and carbon nanotubes [321,322], can be incorporated with hydrogels to make electrically conductive scaffolds. These scaffolds are crucial for cartilage tissue engineering as they mimic the physical properties of native tissue and respond to electrical stimulation. Distler et al. developed a 3D-printable hydrogel combining oxidized Alg–Gel (ADA-Gel) with polypyrrole:polystyrenesulfonate (PPy:PSS) (Fig. 13A) [323]. Hydrogels with 0.1 M PPy exhibited the best mechanical properties and conductivity, similar to native cartilage. Although the PPy-modified hydrogel slightly reduced ATDC5 cell (pre-chondrogenic cell line) attachment and proliferation due to increased stiffness, 3D-printing improved cell seeding efficiency throughout the scaffold.

Liu et al. studied the effects of electrical and mechanical stimulation on cartilage tissue formation in a scaffold made of the conductive polymer PEDOT:PSS and GOPS. They found that electrical stimulation promotes MSC chondrogenesis, while mechanical stimulation enhances MSC chondrogenic differentiation (Fig. 13B) [324]. Another study showed that electric fields enhance Col II and aggrecan expression in human chondrocytes under hypoxia, with a similar but weaker effect in BMSCs and co-cultures [325].

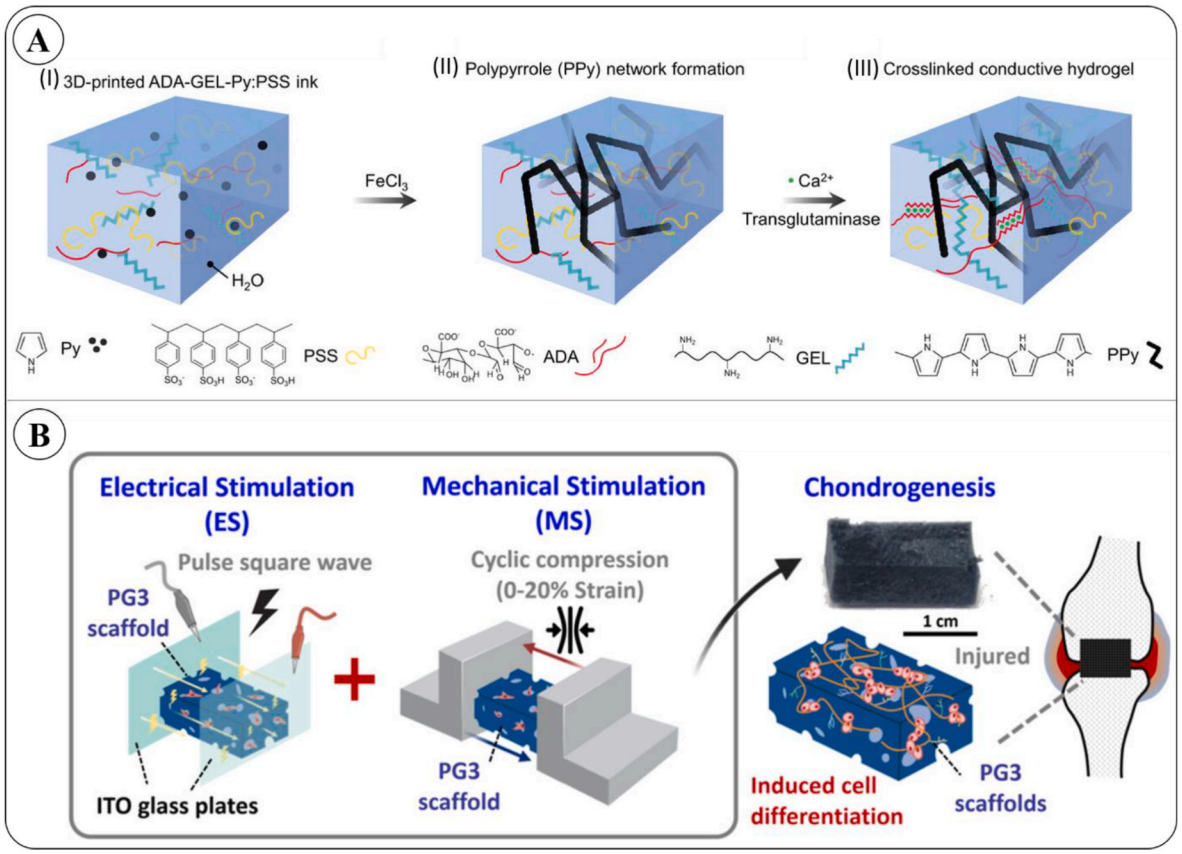

Fig. 13. A) Schematic illustration of ADA-Gel-PPy:PSS conductive hydrogel formation. I) ADA-Gel hydrogel precursor with varying Py and PSS molarities is made. II) After 3D-printing, PPy is formed by oxidizing Py in $FeCl_3$ solution. III) Final ADA-Gel-PPy:PSS scaffolds are cross-linked with Ca^{2+} and microbial transglutaminase to crosslink ADA and Gel network in the hydrogel. Reproduced with permission [323]. Copyright 2024, John Wiley and Sons. B) Schematic illustration of devices for electrical and mechanical stimulation on PEDOT:PSS scaffolds with GOPS (3 wt %, PG3) and scaffold illustration for chondrogenesis. Reproduced (adapted) with permission [324]. Copyright 2023, American Chemical Society.

4.2.3. *Electromagnetic stimuli*

Electromagnetic stimuli in hydrogel design for cartilage involve the controlled application of electromagnetic fields or waves to affect the properties and behavior of the hydrogel and the cells within it. The purpose of electromagnetic stimuli is to modulate cellular activities within the hydrogel to induce specific responses that favor CR [326,327]. As a safe, non-invasive biophysical strategy with minimal or no side effects for tissue repair, electromagnetic fields could not only benefit cartilage-related cells *in vitro* by promoting proliferation, increasing anabolic activities, and antagonizing the catabolic effects of inflammation, but could also provide chondro-protective and chondro-regenerative effects on AC *in vivo* [328,329]. Compared to other biophysical stimulations, electromagnetic fields offer unique advantages as a non-contact non-invasive stimulation. Moreover, compared to optical, acoustic and electrical fields as well as mechanical forces, electromagnetic fields have advantages because of their large force output, high precision and deep tissue penetration [330]. During the preparation of tissue engineered constructs *in vitro*, electromagnetic field treatment offers chondro-inductive effects without any increased risk of contamination [331]. These stimuli can influence the properties and behavior of the hydrogels and the cells within them in different aspects, such as.

- Enhancing the formation of cartilage tissue from stem cells encapsulated in hydrogels;
- Electromagnetic stimuli can also be used to create ordered structures in magnetic hydrogels, which can improve their mechanical strength and biocompatibility [305,332];
- Electromagnetic stimuli are one type of intelligent stimuli that can make hydrogels responsive to the environment [333].

Several groups have reported that an electromagnetic field promotes cartilage formation *in vitro* and *in vivo*. Li et al. developed an injectable adhesive hydrogel with self-healing properties, consisting of Alg-dialdehyde/acrylated β-cyclodextrins/Gel (Alg-DA/Ac-β-CD/Gel) (Fig. 14) [334]. This hydrogel featured both physical and chemical cross-linking as well as self-healing mechanisms. The researchers conducted a comprehensive evaluation of the hydrogel, assessing parameters such as gelation time, swelling ratio, biodegradability and biocompatibility. Their findings indicated the hydrogel's potential for cartilage repair. To enhance versatility, a pre-gel state was established before photo-cross-linking, allowing the hydrogel to accommodate various delivery methods and adapt to diverse clinical scenarios. In further experimentation, the hydrogel was applied in a rat osteochondral defect model, coupled with pulsed electromagnetic fields. The results demonstrated that PEMF not only enhanced the quality of engineered chondrogenic constructs *in vitro* but also promoted chondrogenesis and facilitated cartilage repair *in vivo*.

In general, magnetic hydrogels are composed of a hydrogel matrix and a magnetic component. A variety of magnetic NPs (MNPs) have been incorporated into hydrogel networks, such as γ-Fe_2O_3, Fe_3O_4, and transition metal ferrite NPs ($CoFe_2O_4$, $MnFe_2O_4$, etc.) [335–337]. MNPs offer the advantage of applying remote magnetic-induced physical stimulation, allowing for targeted treatment. In studies involving hMSCs, exposure to a static magnetic field and magnetic-derived shear stress via MNPs resulted in higher chondrogenic differentiation efficiency [338]. In another innovative approach, MNPs-vesicle assemblies

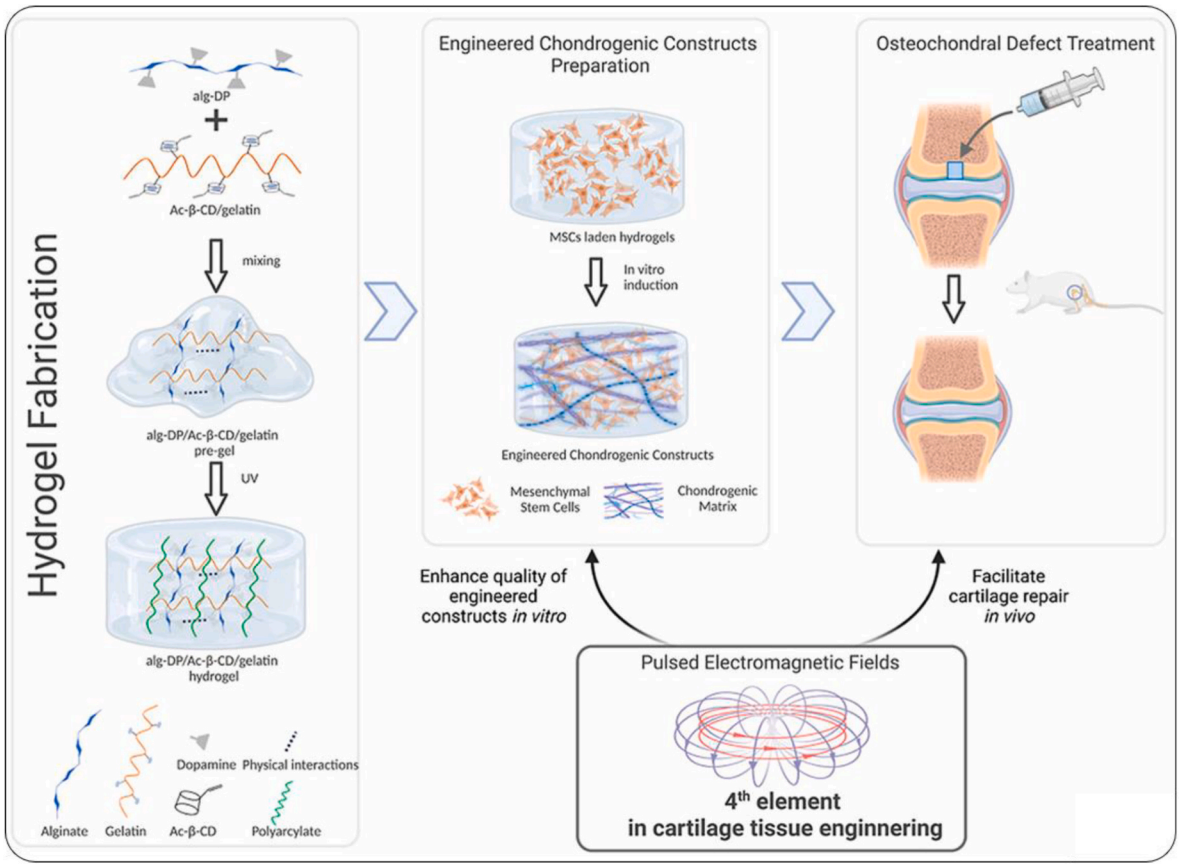

Fig. 14. Schematic illustration of Alg-DA/Ac-β-CD/Gel hydrogels design and pulsed electromagnetic field use *in vitro* and *in vivo*. Reproduced under the terms of the CC BY-NC-ND 4.0 license [334]. Copyright 2023, The Authors, published by Elsevier.

were created by cross-linking phospholipid vesicles and MNPs. When immobilized with chondrocytes within a calcium Alg hydrogel, this biomaterial responded to alternating magnetic fields by translating non-invasive magnetic signals into cellular responses. Notably, the chondrocytes in the gel responded to the magnetic release of ascorbic acid-2-phosphate (AAP), applied as an additive, by producing a high level of Col [339]. Similarly, DEX-coated MNPs integrated into distinct layers of agarose constructs formed trilayered ferrogels. The application of an external magnetic field resulted in increased sGAG content over time in bovine chondrocytes seeded within the ferrogels. These findings collectively underscore the promising potential of electromagnetic stimuli in hydrogel design for CR [334].

As stated, one of the key aspects of electromagnetic actuation is the fabrication of smart hydrogels. These stimuli can control the drug release, shape change, alignment, or differentiation of the hydrogel and the cells within it. For example, Wang et al. developed a smart hydrogel based on PNiPAAm and iron oxide NPs, which had a thermo-responsive and magnetic-responsive behavior [340]. Chen et al. fabricated hydrogel scaffolds that incorporated cellulose nanocrystals and SF and labeled them with ultrasmall superparamagnetic iron oxide (USPIO) [341]. This scaffold enhanced chondrogenic gene expression *in vitro* and AC tissue formation and also tracked scaffold degradation during the articular CR process.

In summary, we have reviewed the recent advances in the use of biophysical stimuli on the hydrogels for cartilage tissue repair, which are summarized in Table 7.

These stimuli are essential for optimizing hydrogel design and improving clinical outcomes in cartilage tissue engineering. Biochemical stimuli enhance cellular activities such as proliferation and differentiation, although controlled spatiotemporal release remains a challenge. Advanced techniques like 4D-bioprinting and gene therapy could further enhance the efficacy of chemical signal-based approaches. Biophysical stimuli, particularly mechanical forces, improve hydrogel mechanical properties and stimulate cartilage-specific matrix production. Electrical and electromagnetic stimuli further enhance cellular behavior and tissue regeneration, offering non-invasive methods to replicate native cartilage environments. However, while these advancements are promising, further research is needed to optimize application parameters and ensure the long-term stability and safety of these hydrogel systems *in vivo*. The potential for combining electrical and electromagnetic stimuli with other biophysical cues, such as mechanical forces, also warrants exploration to create more holistic and effective strategies for cartilage repair.

5. Future perspectives and challenges

Advancements in the field of designing hydrogels for articular CR have shown great promise, but there are still important future perspectives and challenges that need to be addressed. This section discusses several areas of focus that can drive the field forward and overcome existing limitations.

One area of future exploration lies in the advancement of biomaterial design. Researchers can continue to investigate and develop novel biomaterials with improved properties for hydrogel-based CR. These materials may possess enhanced mechanical strength, improved biocompatibility and the ability to provide controlled release of bioactive factors, such as GFs or cytokines. Additionally, the utilization of advanced fabrication techniques, such as nanotechnology or 4D-bioprinting, can enable the creation of precise and customizable hydrogel structures that closely mimic the native cartilage environment. Bioinspired approaches hold significant potential for future advancements in hydrogel design. By incorporating bioinspired cues into hydrogel

Table 7
A summary of biophysical stimuli on the hydrogels for cartilage tissue repair.

Hydrogel	Type of stimulation	Duration/Condition	Results	References
Agarose	Mechanical (compression)	1 h/day, 28 days	• Enhance Agc and Col 2a1 expression • Inhibit hypertrophy markers such as Col 1a1, Col 10a1, MMP-13, and RUNX2	[342]
PANa	Electrical	20 days	• Ionic conductivity of 1.65 S m−1 • Excellent mechanical strength with a fracture stress of over 7 MPa	[343]
Gel, CS, HA, and Ch	Mechanical (cyclic dynamic compressive, 20 % strain)	3 h/day, 14 days, 1 Hz	• Enhance neo-cartilage formation in dynamic culture during subcutaneous implantation in nude mice	[344]
Fibrin	Mechanical (Static and perfusion bioreactor)	3 weeks, 2.5 μL/s	• Integration with native tissue as a result of preimplantation bioreactor perfusion treatment • Increase secretion of cartilage matrix components	[345]
Gel, β-cyclodextrin, GPTMS, and Fe3O4 MNPs	Electromagnetic (constant static magnetic field)	100 mT	• Promote the differentiation of BMSCs into cartilage • Strong regenerative effect of magnetic hydrogel and BMSCs combined with pulsed electromagnetic field in the results of animal experiments.	[346]
HA, and Gel	Electrical	30 min every 6 h/day, 21 days with 10 mV/cm at 60 kHz	• Increase the mechanical properties of hydrogels • Improve the morphology of the cells inside the stimulated hydrogels toward roundness • Enhance Sox9, and Agc • Increase normalized content of GAGs and Col to DNA in stimulated samples	[347]
Starch, PCL, Fe_3O_4, MNPs	Electromagnetic	0–5 T	• Promote cellular differentiation	
Ch-BGP, and Opy	Electrical	–	• Decrease pore size, and increase gelation time, swelling ratio, conductivity and degradation time with increasing content OPy • A significant increase in scaffold conductivity to 1.9 S m^{-1} with the addition of OPy (close to the value reported for native cartilage)	[348]
PEG, CS-SH, Genscript, and Irgacure 2959	Mechanical (intermittent unconfined dynamic compressive strains applied at 5 % peak to peak strain)	1 h/day, 3 weeks, 1 Hz with 23 h of rest under a tare strain of <0.1 %	• Dynamic loading and TGF-β3 synergistically induced stable chondrogenesis and prevented hypertrophy • Promotion of a stable chondrogenic phenotype (Agc + Col II) with reduced Col X by dynamic loading with TGF-β3	[349]
Fibrin, and HA	10 %/0.5 Hz compression, 25°/0.5 Hz rotation	1 h/day, 14 days	• sGAG/DNA upregulation in loaded samples both in the presence or absence of fibroblast growth factor-18 variant	[350]
Fibrin	Mechanical (1 Hz to an amplitude of 10 % strain, after a 0.01 N preload)	2 h/day, 5 days per week/ 2 weeks	• Increase mRNA expression of Col 2a1 and ACAN genes after more than 2 weeks of priming samples • Increase Col 2a1 and Col 10a1 and decrease Sox9 expression due to immediate mechanical loading • Increase of GAG and Col deposition and mechanical properties due to priming time	[351]
PLGA, PEG, and PLGA	Electromagnetic (sinusoidal)	4 h/day, 15 Hz, 1 mT	• Enhance proliferation and chondrogenic differentiation of BMSCs by activating PI_3K/AKT/mTOR and Wnt_1/LRP_6/ β-catenin signaling pathways under the influence of electromagnetic field • Increase repair of osteochondral defects in rabbits and especially cartilage repair by scaffolds	[352]
HA, Gel, and SPIOs NPs	Electromagnetic (sinusoidal)	1 h/day, 5 days, 0.22 T	• Enhance sGAG synthesis through magnetic stimulation • Upregulate expression of cartilage-specific genes	[338]

Abbreviations: PANa: PVA sodium phytate, MMP13: matrix metalloproteinase-13, RUNX2: Runt-related transcription factor2, iPSCs: induced pluripotent stem cells, Ch-BGP: chitosan-β-glycerophosphate, Opy: oligopyyrole, CS-SH: thiolated chondroitin sulfate, SPIOs: superparamagnetic iron oxide.

scaffolds, researchers can aim to enhance chondrogenic differentiation and tissue development. This may include incorporating ECM-mimicking components or providing mechanical stimulation to guide cell behavior and promote tissue organization. Exploring topographical cues or biochemical gradients within hydrogel constructs can further enhance the functionality and regenerative potential of the engineered cartilage tissue. The integration of stem cells and tissue engineering is another area that warrants attention. Understanding the intricate interactions between hydrogels and stem cells is crucial for optimizing hydrogel properties to support stem cell viability, proliferation, and differentiation towards the chondrogenic lineage. Researchers can explore strategies to improve the retention, survival and functionality of transplanted stem cells within hydrogel-based constructs, thereby enhancing the overall regenerative capacity of the engineered cartilage. Personalized medicine and patient-specific approaches are also important for future advancements in hydrogel design. Considering individual variations in cartilage properties or disease characteristics can help tailor hydrogel-based therapies to specific patients. The integration of imaging techniques and computational modeling can aid in the development of patient-specific hydrogel constructs, thereby optimizing treatment outcomes and enhancing patient satisfaction. Translation to clinical applications remains a critical challenge. While preclinical studies have shown promising results, further research is required to evaluate the long-term safety, efficacy and functional outcomes of hydrogel-based approaches in clinical settings. The development of robust regulatory frameworks and standardized protocols is essential to facilitate the clinical translation of hydrogel-based CR therapies and ensure their successful implementation. Overcoming challenges associated with hydrogel degradation, stability and long-term performance in the dynamic joint environment is another important area of focus. It is essential to enhance hydrogels' mechanical characteristics to meet the strict specifications of load-bearing joints. Additionally, addressing immunological responses and inflammatory reactions to synthetic hydrogel materials is necessary to ensure successful integration and long-term viability of the engineered cartilage tissue. Collaborative and multidisciplinary approaches will play a pivotal role in driving future

advancements in hydrogel design for articular CR. Encouraging collaboration among scientists, engineers, clinicians and regulatory bodies can foster the exchange of knowledge and expertise from various fields. This interdisciplinary research approach will accelerate the development and translation of hydrogel-based therapies, bringing us closer to effective treatments for cartilage defects and improving the quality of life for individuals suffering from articular cartilage-related conditions. To summarize, exploring future perspectives and addressing existing challenges in the design of hydrogels for articular CR is crucial for advancing the field. Through advancements in biomaterial design, bioinspired approaches, integration of stem cells, personalized medicine, clinical translation, overcoming challenges and with collaborative efforts, researchers can pave the way for the development of effective and biomimetic hydrogel-based strategies that restore the structure, function and biomechanical properties of articular cartilage.

6. Conclusion

The hydrogel design for articular CR holds great promise as an advanced approach to addressing the limitations of traditional treatments for cartilage defects. Hydrogels, with their unique properties, are promising scaffolds for cartilage tissue engineering. They offer a suitable 3D microenvironment that maintains cell shape and function while facilitating cell-cell and cell-matrix interactions. By understanding and optimizing the design parameters, such as type of material, mechanical properties, biodegradability, porosity and interconnectivity, and integration capability with the adjacent native tissue, scientists can create hydrogels that effectively restore the structure, function and biomechanics of articular cartilage. Moreover, the design of stratified-structural hydrogels that mimic the native cartilage structure and the application of environmental stimuli (such as biophysical and biochemical cues) is essential for achieving optimal CR results. These innovations can significantly enhance the quality of life for people suffering from cartilage defects and related conditions, paving the way for more successful and biomimetic cartilage tissue engineering approaches.

Ethics approval and consent to participate

Ethics approval and consent to participate do not apply to this review manuscript.

CRediT authorship contribution statement

Fariba Hashemi-Afzal: Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hooman Fallahi:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Conceptualization. **Fatemeh Bagheri:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Maurice N. Collins:** Writing – review & editing, Conceptualization. **Mohamadreza Baghaban Eslaminejad:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Hermann Seitz:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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