Materials Today Bio 31 (2025) 101623

Contents lists available at ScienceDirect

Materials Today Bio

journal homepage: www.journals.elsevier.com/materials-today-bio

Dynamic Col-HZ Hydrogel with efficient delivery of bioactivator promotes ECM deposition and cartilage formation

Honglei Wang [a,b,1], Xu Wu [a,b,1], Lili Chen [a,b], Hua Tong [a,b], Xuerui Hu [a,b], Aijuan He [a,b], Chenlong Li [a,b], Xudong Guo [c,*], Yaoyao Fu [a,b,**], Tianyu Zhang [a,b,d,***]

[a] Department of Facial Plastic and Reconstructive Surgery, Eye & ENT Hospital, Fudan University, Shanghai, 200031, China
[b] ENT Institute, Eye & ENT Hospital, Fudan University, Shanghai, 200031, China
[c] Clinical and Translational Research Center of Shanghai First Maternity and Infant Hospital, Shanghai Key Laboratory of Maternal Fetal Medicine, Frontier Science Center for Stem Cell Research, National Stem Cell Translational Resource Center, School of Life Sciences and Technology, Tongji University, China
[d] NHC Key Laboratory of Hearing Medicine, Fudan University, Shanghai, 200031, China

ARTICLE INFO

Keywords:
Dynamic hydrogel
Chondrocyte
Drug delivery
Matrix deposition
Cartilage formation

ABSTRACT

Efforts in cartilage tissue engineering to repair injuries have seen limited success, primarily due to the inability of scaffold materials to establish a microenvironment conducive to extracellular matrix (ECM) deposition by chondrocytes. Hydrogels, which mimic human tissue, are commonly employed as scaffold materials; however, their constrained network structure and low bioactivity impede chondrocyte ECM deposition, complicating cartilage repair. In this study, we developed dynamic Col-HZ hydrogels featuring adaptive networks by forming hydrazone (HZ) bonds between bioactive natural collagen and synthetic polyethylene glycol (PEG). In contrast to static hydrogels that rely on covalent bonds, Col-HZ dynamic hydrogels facilitate chondrocyte migration and ECM deposition. Additionally, the aldehyde groups on the Col-HZ hydrogel scaffold can engage in dynamic Schiff base bonding with amine groups. Leveraging this non-covalent interaction, we incorporated the bioactivator TD-198946, known to enhance ECM synthesis, into the Col-HZ hydrogel. This significantly boosted ECM deposition and reduced inflammation. Transcriptomic sequencing and bioinformatics analyses indicate that both the dynamic network of the hydrogel and the binding of TD-198946 promote cartilage ECM deposition through modulation of the Wnt/β-catenin signaling pathway. Consequently, the Col-HZ dynamic hydrogel, in combination with TD-198946, creates an improved microenvironment that supports ECM deposition and facilitates cartilage tissue formation.

1. Introduction

Cartilage defects resulting from trauma, tumors, infections, and congenital diseases (e.g., in the joints, nose, ears, larynx, and trachea) have a significant impact on patient health and present substantial challenges in clinical treatment [1]. According to materiobiology theory, creating a microenvironment conducive to cell survival through materials can activate the cellular repair potential, thereby promoting tissue regeneration and repair [2,3]. In cartilage development and maintenance, the matrix secreted by chondrocytes plays a pivotal role [4]. However, the microenvironment provided by current scaffold materials often fails to effectively promote cartilage matrix deposition, limiting the success of regeneration and repair. Thus, there is an urgent need for material systems that enhance cartilage matrix deposition.

Hydrogels, composed of hydrophilic polymers, form three-dimensional porous networks that closely resemble human tissue, making them ideal scaffolds for supporting cell survival and function [5,6]. Their tunable physicochemical properties have led to widespread applications in tissue regeneration [7]. In comparison to static hydrogels formed by fixed chemical bonds, dynamic hydrogels created through

* Corresponding author. School of Life Sciences and Technology, Tongji University, Shanghai, 200092, China.
** Corresponding author. Department of Facial Plastic and Reconstructive Surgery, Eye & ENT Hospital, Fudan University, Shanghai, 200031, China.
*** Corresponding author. ENT Institute, Eye & ENT Hospital, NHC Key Laboratory of Hearing Medicine, Fudan University, Shanghai 200031, China.
E-mail addresses: 19504@tongji.edu.cn (X. Guo), fuyaoyao2007@126.com (Y. Fu), ty.zhang2006@aliyun.com (T. Zhang).
[1] Honglei Wang and Xu Wu contribute equally.

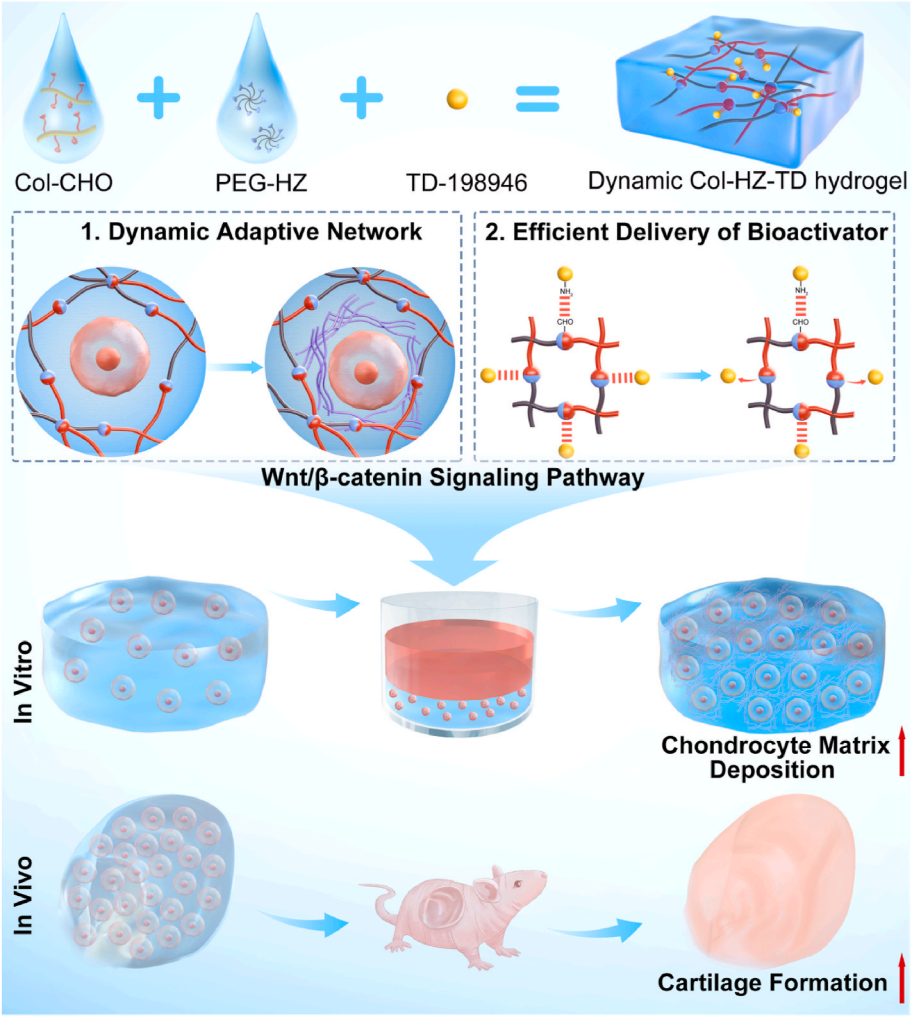
https://doi.org/10.1016/j.mtbio.2025.101623
Received 16 January 2025; Received in revised form 17 February 2025; Accepted 27 February 2025
Available online 28 February 2025

dynamic bonds offer advantages for tissue regeneration by mimicking the dynamic microenvironment of the natural extracellular matrix (ECM), thus accommodating cellular behaviors such as proliferation and differentiation [8–10].

Recent research has focused on the development of novel dynamic hydrogels and their effects on cell behavior. For instance, David J. Mooney and colleagues developed calcium ion-crosslinked alginate dynamic hydrogels that enhance diffusion, proliferation, and osteogenic differentiation of mesenchymal stem cells [11]. Liming Bian and colleagues designed dynamic hydrogels with host-guest interactions that promote mechanotransduction and osteogenic differentiation of stem cells in a three-dimensional context [12]. Additionally, dynamic hydrogels possess shear-thinning and self-healing properties, which enable them to adapt to the complex mechanical environment of cartilage [13]. However, many existing dynamic hydrogels require complex functionalization processes and specific conditions (e.g., calcium ions or redox agents) to exhibit their dynamic properties. These requirements can lead to chondrocyte damage, matrix degradation, or cartilage calcification, limiting their application in cartilage regeneration. Therefore, the development of new dynamic hydrogels that foster a three-dimensional microenvironment conducive to chondrocyte matrix deposition is essential.

In natural ECM, protein or polysaccharide components can bind growth factors and transmit their active signals to cells, thereby regulating cellular behavior [14,15]. By mimicking this process, active signaling molecules (e.g., growth factors or bioactive drugs) can be incorporated into hydrogels to promote cartilage matrix deposition [16–18]. TD-198946, a small-molecule chondrogenic inducer, upregulates SOX9 expression, inhibits hypertrophy, and enhances ECM deposition, making it a promising candidate for cartilage regeneration [19,20]. Additionally, its rapid efficacy and low cost make it well-suited for hydrogel-based delivery in cartilage repair [21]. However, the method of immobilizing the active molecule significantly impacts their efficacy. While covalent grafting can prevent rapid diffusion, this non-selective fixation may disrupt molecular structures and fail to optimally regulate cell behavior [22,23]. In contrast, the use of reversible bonds for delivering active molecules better preserves their bioactivity, reduces dosage, enhances therapeutic effects, and minimizes potential side effects [24].

Based on the above considerations, we developed Col-HZ dynamic hydrogels with adaptive networks by forming hydrazone (HZ) bonds between collagen and polyethylene glycol (PEG) (see Scheme 1). In contrast to static hydrogels based on covalent bonds, Col-HZ hydrogels promote chondrocyte migration and matrix deposition. Moreover, the aldehyde groups in Col-HZ hydrogels can dynamically bind the bioactive molecule TD-198946 via Schiff base bonds, resulting in Col-HZ-TD hydrogels that significantly enhance chondrocyte matrix deposition. Transcriptomic sequencing and bioinformatics analyses suggest that the dynamic adaptive network of Col-HZ hydrogels, in conjunction with TD-198946, promotes matrix deposition by modulating the Wnt/β-catenin signaling pathway. In vivo experiments using subcutaneous cartilage formation models in nude mice confirmed the favorable effects of Col-



Scheme 1. Dynamic Col-HZ hydrogel with presentation of TD-198946 is fabricated to create a microenvironment that promotes cartilage matrix deposition.

HZ dynamic hydrogels on cartilage formation. Therefore, the Col-HZ dynamic hydrogel system combined with TD-198946 provides an improved microenvironment that promotes cartilage matrix deposition and facilitates cartilage tissue formation.

2. Result & discussion

2.1. Preparation and characterization of Col-HZ Hydrogel

We selected recombinant human collagen type I, a natural macromolecular protein, and polyethylene glycol (PEG), a synthetic polymer, as the key components for hydrogel formation. Despite Collagen II being the main component of native cartilage, Collagen I was chosen for its superior mechanical stability, bioactivity, and processability. Collagen I can form robust fibrillar networks with superior mechanical integrity, essential for scaffold stability in the early stages of repair to support cell growth and tissue regeneration [25,26]. Compared to type II collagen, type I collagen contains more functional cell-binding sites, which enhance mesenchymal stem cell (MSC) adhesion and chondrogenic differentiation, promote chondrocyte proliferation, and suppress chondrocyte dedifferentiation in the short term [27,28]. Additionally, Collagen I is more readily available and cost-effective, making it an optimal choice for various cartilage repair scaffolds (e.g. Chondro-Gide®, Geistlich Pharma AG; Novocart® Basic, TETEC AG; MeRG®, Bioteck Srl) [29,30]. PEG, as another component, offers advantages such as excellent biocompatibility, high water solubility, a controllable molecular structure, and ease of functional modification [31].

As shown in Fig. 1A, Col-HZ dynamic hydrogels were prepared by reacting Col-CHO with 8-arm-PEG-Hy at physiological pH and temperature. Initially, collagen was mixed with SC-PEG-CHO (Mw = 2000) to undergo an amidation reaction at 37 °C, yielding aldehyde-modified collagen (Col-CHO). This was then mixed in equal proportions with an 8-arm-PEG-Hy solution and thoroughly homogenized for further characterization and testing. The formation of hydrazone bonds within the hydrogel was confirmed by infrared spectroscopy, with the FTIR spectrum displaying a new peak for the C=N hydrazone bond at 1630 cm^{-1}, validating bond formation (Fig. S1A, Supporting Information). The gelation time, determined using an inverted vial test, revealed that a transparent hydrogel formed in approximately 6 min after thorough mixing (Fig. 1B). After freeze-drying, scanning electron microscopy revealed a porous microstructure within the Col-HZ hydrogel (Fig. S1B, Supporting Information). As shown in Fig. 1D, the Col-HZ dynamic hydrogel exhibited injectable properties, enabling the hydrogel precursor solution to be injected into an auricle-shaped mold, retaining its shape after manipulation with tweezers. The self-healing property of the hydrogel is essential for the subsequent construction of auricular cartilage.

The aldehyde groups on Col-CHO react with the hydrazone functional groups of 8-arm-PEG-Hy under the catalyst 4-amino-phenylalanine (4a-Phe), forming hydrazone bonds that constitute the hydrogel network. Hydrazone bonds, dynamic covalent bonds that can reversibly break and reorganize, offer distinct advantages over other dynamic bonds (such as hydrogen bonds, electrostatic interactions, coordination bonds, and disulfide bonds). These advantages include a relatively simple functionalization process, good compatibility, and the ability to undergo dynamic reorganization under mild conditions, without the need for specific ions or oxidizing/reducing agents. Furthermore, the acidic ECM secreted by chondrocytes promotes the opening and formation of hydrazone bonds, rendering the hydrogel network more conducive to matrix deposition by chondrocytes [32].

The macroscopic mechanical properties of Col-HZ hydrogels were assessed using a rotational rheometer [33]. As shown in Fig. 1D, the

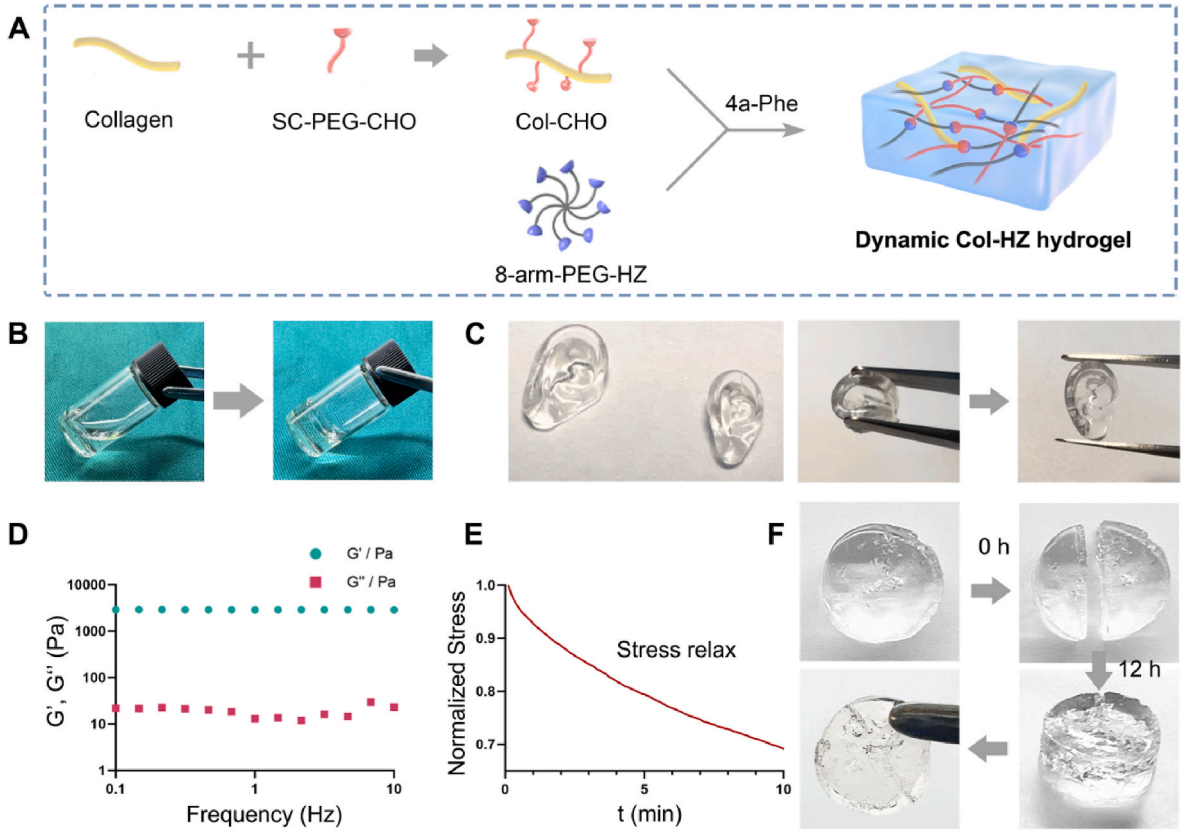


Fig. 1. Preparation and Characteristics of Dynamic Col-HZ Hydrogel. (A) Schematic representation of the preparation process for Col-HZ dynamic hydrogel. (B) Gelation process of the Col-HZ dynamic hydrogel. (C) The Col-HZ dynamic hydrogel can be molded into an auricle shape and demonstrates a degree of toughness. (D) Elastic modulus and loss modulus of the Col-HZ hydrogel. (E) Stress relaxation behavior of the Col-HZ hydrogel. (F) Self-healing capability of the hydrogel.

storage modulus (G′) of the hydrogel is approximately 2500 Pa, while the loss modulus (G″) is around 30 Pa. Stress relaxation behavior, indicative of the unique viscoelasticity of dynamic hydrogels, is demonstrated in Fig. 1E. Under constant strain applied by the rheometer, the internal stress of Col-HZ hydrogels decreases continuously over time, exhibiting classic stress relaxation behavior with a half-life relaxation time of approximately 9 min. The reversible hydrazone bonds confer self-healing capabilities to Col-HZ hydrogels. As demonstrated in Fig. 1F, when the hydrogel was cut in half with a blade and reassembled, it reconnected after 12 h.

To assess the degradation of the hydrogel, we immersed the samples in PBS and incubated them at 37 °C on a shaker, removing them at specified time points for weighing. Upon degradation, the hydrogel's network density decreased, facilitating increased water absorption. Therefore, the swelling behavior was used as a key indicator of hydrogel degradation. Despite being fully submerged in an aqueous solution and subjected to the vigorous shaking environment, both hydrogels retained their shape after 14 days. As shown in Fig. S2, the Col-HZ-TD hydrogel exhibited a higher swelling ratio compared to the static GelMA hydrogel at days 3, 7, and 14. This enhanced swelling is likely attributed to the loosening of the Col-HZ-TD network, which results from the opening of dynamic hydrazone bonds.

2.2. *Dynamic network of Col-HZ Hydrogel is Beneficial for Chondrocyte Migration*

Chondrocytes, as the essential components of cartilage tissue, play a critical role in cartilage development and regeneration through their behaviors [34,35]. During cartilage formation, chondrocytes migrate to secrete the ECM and differentiate into mature cells, facilitating normal skeletal and joint formation. After cartilage injury, the migratory capacity of chondrocytes is crucial for repairing damaged areas and generating new cartilage tissue. Moreover, migrating chondrocytes can sense mechanical signals, regulating matrix synthesis and deposition to maintain cartilage function and structure [36].

Dynamic hydrogels sustain macroscopic mechanical strength while accommodating cellular movement through microscopic network alterations [37,38]. To investigate the effects of Col-HZ hydrogel on chondrocyte migration, we performed a migration experiment. As illustrated in Fig. 2A, hydrogel precursor solutions loaded with chondrocytes were injected into a 48-well plate, resulting in a uniform distribution of chondrocytes upon gelation. The plate was then inverted, and a line was drawn in the center of each well (red line). The right side of the hydrogel was entirely removed along the marked line using a spoon, and the space was refilled with cell-free hydrogel precursor solution. Live-dead staining was performed after 7 and 14 days to assess cell migration (blue line).

Live-dead staining results indicated that chondrocytes encapsulated in the hydrogel remained viable, demonstrating excellent cell compatibility (Fig. 2C). The migration distance of chondrocytes was quantified (Fig. 2D), using the cell positions on day 1 as a baseline. By day 7, both the dynamic Col-HZ hydrogel and Col-HZ-TD hydrogel (delivering TD-198946) exhibited significant chondrocyte migration, with cells reaching the furthest distance visible by day 14. In contrast, the migration distance of chondrocytes in the static GelMA hydrogel was significantly lower at both days 7 and 14, indicating that the dynamic scaffold

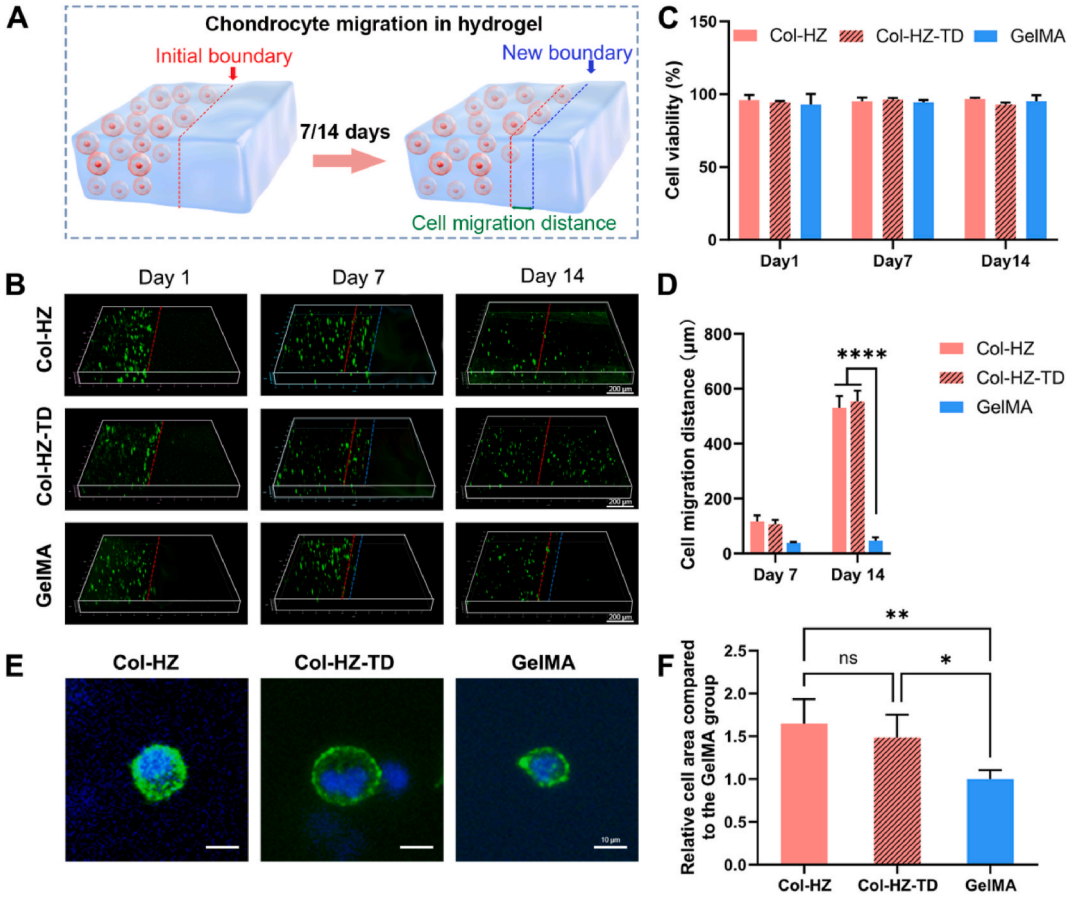


Fig. 2. Col-HZ Hydrogels are Beneficial for Chondrocyte Migration. (A) Schematic diagram of the chondrocyte migration experiment in hydrogels. The red dashed line indicates the initial cell boundary, while the blue dashed line represents the cell boundary after culture. (B) Live-dead staining indicating cell positions. (C) Quantification of cell viability. (D) Quantitative results of cell migration distance. (E) Morphology of chondrocytes within the hydrogel. (F) Quantification of chondrocyte area.

network of the Col-HZ hydrogel effectively supports chondrocyte migration.

Furthermore, we examined the morphology of chondrocytes in three-dimensional culture within the hydrogel and quantified cell area (Fig. 2D and E). The results revealed that, compared to GelMA static hydrogels, chondrocytes in both the Col-HZ and Col-HZ-TD dynamic hydrogel groups exhibited larger spreading areas. Research suggests that increased chondrocyte volume enhances metabolic activity, thereby improving matrix deposition capacity [39,40]. Static hydrogels, such as GelMA, based on covalent crosslinked networks, possess rigid and fixed structures that restrict cellular migration. This configuration imposes spatial constraints, inhibiting free movement and intercellular communication within the matrix. Such restrictions can hinder critical processes for chondrocytes, including migration, matrix remodeling, and responses to mechanical signals, all vital for cartilage repair and tissue formation. In contrast, the Col-HZ hydrogel, constructed with dynamic

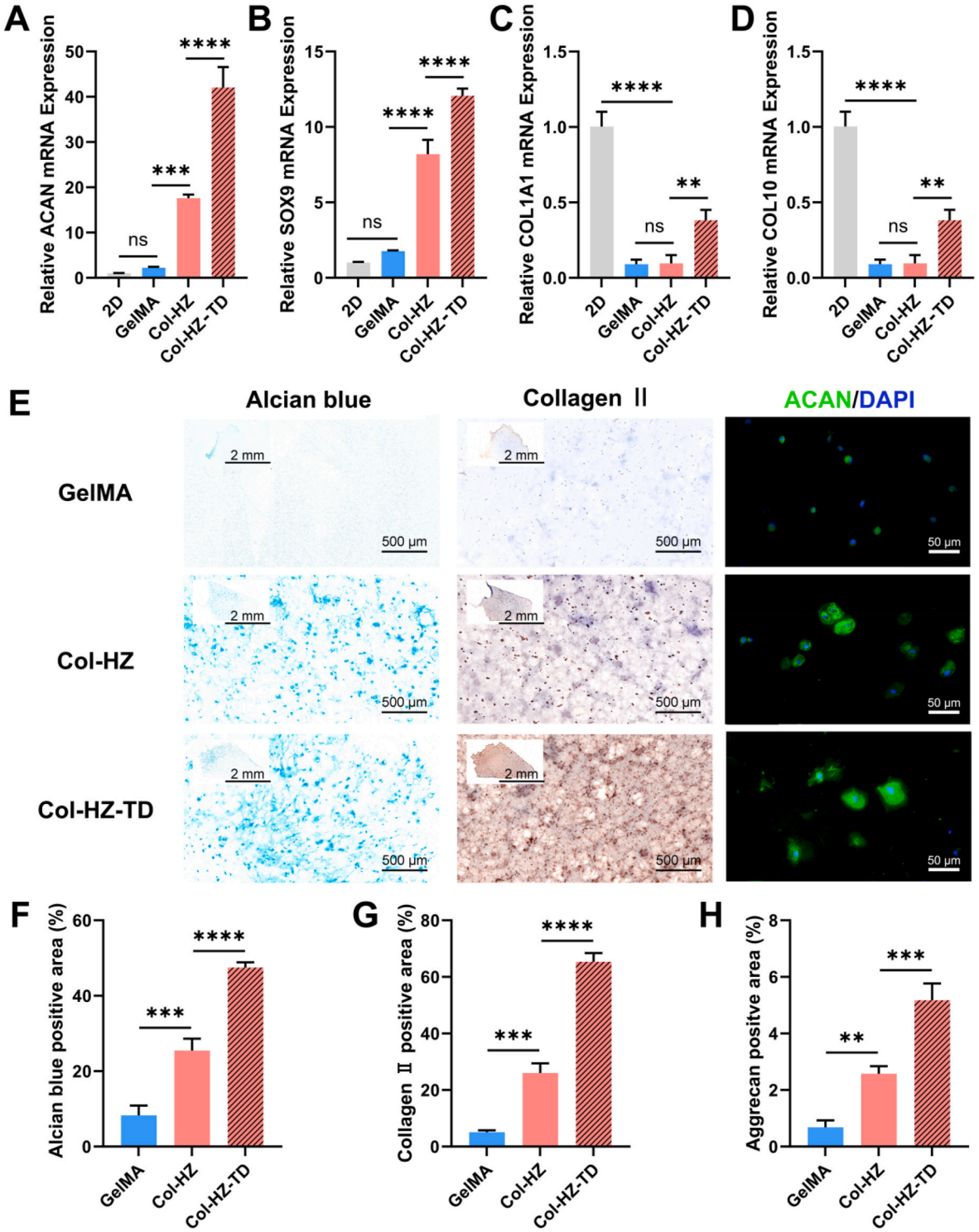


Fig. 3. Col-HZ Hydrogels Promote Chondrocyte Matrix Deposition. (A–D) After 7 days of three-dimensional culture in hydrogels, PCR experiments were conducted to compare gene expression levels in chondrocytes. (E) After 14 days of three-dimensional culture in hydrogels, immunohistochemistry and immunofluorescence staining were performed to assess chondrocyte matrix deposition. (F–H) Quantitative results of immunohistochemistry and immunofluorescence staining.

hydrazone bonds, offers an adaptive three-dimensional environment that promotes chondrocyte migration.

2.3. Col-HZ dynamic hydrogels synergistically deliver TD-198946 to promote Chondrocyte matrix deposition

Matrix deposition by chondrocytes is critical for cartilage regeneration and repair [41]. Cartilage is primarily composed of chondrocytes and the extracellular matrix (ECM) they produce, which includes collagen and proteoglycans. The ECM provides structural support, imparting strength and elasticity to cartilage while preserving its morphology and function [42]. Furthermore, it creates a conducive microenvironment for chondrocytes, with matrix components interacting with receptors on chondrocyte surfaces to regulate key cellular behaviors such as proliferation, differentiation, and matrix synthesis, all of which are vital for cartilage maintenance and repair [43]. However, the limited capacity of chondrocytes to secrete matrix in the human body presents a significant challenge for effective cartilage regeneration.

Enhancing chondrocyte matrix deposition through biomaterials has become a central focus in cartilage regeneration research [44,45]. As demonstrated in Fig. 2, Col-HZ dynamic hydrogels offer a three-dimensional environment that supports chondrocyte survival and migration. We investigated the impact of these hydrogels on matrix deposition by encapsulating primary chondrocytes in Col-HZ, Col-HZ-TD dynamic hydrogels, and GelMA static hydrogels, comparing these to two-dimensional (2D) cultures.

As shown in Fig. 3A–D, after 7 days of culture, gene expression was assessed via PCR, analyzing cartilage-specific gene ACAN, key regulatory gene SOX9, fibrosis-related gene COL1A1, and hypertrophy gene COL10. Both the Col-HZ and Col-HZ-TD dynamic hydrogel groups significantly upregulated ACAN and SOX9 expression compared to the GelMA static hydrogel and 2D culture groups, with the Col-HZ-TD group showing superior results. Thus, Col-HZ dynamic hydrogels, in combination with TD-198946 delivered via Schiff base interactions, synergistically enhance chondrocyte matrix deposition. Additionally, three-dimensional culture using Col-HZ, Col-HZ-TD dynamic hydrogels, and GelMA static hydrogels downregulated COL1A1 and COL10 expression compared to 2D cultures.

Subsequently, as illustrated in Fig. 3E, matrix deposition by chondrocytes was evaluated after 14 days of culture using immunohistochemistry and immunofluorescence staining. Quantitative results shown in Fig. 3F and G reveal that Alcian blue staining and immunohistochemical staining for type II collagen exhibited greater blue-stained cartilage matrix in the Col-HZ and Col-HZ-TD dynamic hydrogel groups compared to the GelMA static hydrogel group, with the Col-HZ-TD group showing the most pronounced results. Furthermore, immunofluorescence staining demonstrated that chondrocytes in both Col-HZ and Col-HZ-TD dynamic hydrogel groups exhibited larger spreading areas and, as quantified in Fig. 3H, secreted more ACAN matrix compared to the GelMA static hydrogel group.

2.4. Mechanistic study of Col-HZ dynamic hydrogels enhancing Chondrocyte matrix deposition

Compared to two-dimensional cultures, three-dimensional (3D) chondrocyte culture using hydrogels offers multiple advantages [46,47]. Hydrogels can mimic the 3D architecture of the ECM, providing a supportive growth environment akin to natural cartilage tissue, thus promoting substantial ECM deposition by chondrocytes [48]. Dynamic hydrogels, formed with reversible physical or chemical bonds, more effectively simulate the natural ECM than static hydrogels [10,49]. Despite this, research on dynamic hydrogels regulating chondrocyte behavior and in-depth ECM studies remains limited. The Col-HZ dynamic hydrogel was designed to promote chondrocyte matrix deposition, and transcriptome sequencing was performed to investigate its mechanisms.

Chondrocytes were encapsulated in Col-HZ dynamic hydrogels and GelMA static hydrogels, and after 7 days, RNA was extracted for bulk RNA sequencing analysis. Initial clustering based on gene expression in the Col-HZ and GelMA groups revealed significant differences (Fig. S3A, Supporting Information). Genes upregulated in Col-HZ but downregulated in GelMA were classified into Group C1, while genes downregulated in Col-HZ but upregulated in GelMA were placed in Group C2 (Fig. S3B, Supporting Information).

As shown in Fig. 4A, a volcano plot illustrates the differentially expressed genes (DEGs) between the two groups, highlighting significant genes (adjusted p-value <0.05, absolute log2 fold-change >1). The results indicated that Col-HZ dynamic hydrogels upregulated 115 genes associated with cartilage matrix deposition (e.g., COL2A1, COL9A2) and downregulated 148 genes linked to inflammation (e.g., MMP1, IL6, S100A9) compared to GelMA. Network interaction analysis confirmed that Col-HZ dynamic hydrogels upregulated genes involved in cartilage matrix deposition and downregulated inflammatory genes (Figs. S3C and D, Supporting Information).

Subsequently, DEGs underwent Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses (Fig. 4B and C). GO analysis revealed that, compared to static hydrogels, Col-HZ dynamic hydrogels enhanced biological processes related to cartilage regeneration and matrix deposition while suppressing inflammation and matrix degradation processes. KEGG pathway analysis indicated that Col-HZ dynamic hydrogels upregulated Wnt signaling pathways and pathways supporting cartilage ECM deposition while downregulating inflammatory pathways. Further network interaction analysis identified WNT7B, a key gene in the Wnt pathway, as a central regulatory hub across multiple pathways (Fig. 4D). Gene Set Enrichment Analysis (GSEA) confirmed a positive correlation between WNT7B, the chondrogenic factor SOX9, and matrix deposition-related COL9A2 (Fig. 4E and F).

Further analysis of transcription factor activity based on differential gene expression revealed significantly upregulated and downregulated transcription factors in Col-HZ dynamic hydrogels relative to GelMA static hydrogels (Fig. 4G). GO enrichment analysis of the significantly upregulated transcription factors suggested that GLI3, SOX9, NR4A2, and SMAD3 may exert transcriptional regulation through interactions with β-catenin, further emphasizing the critical role of the WNT signaling pathway (Fig. 4H).

As shown in Fig. 4I, predicted differentiation direction analysis for Col-HZ dynamic hydrogels and GelMA static hydrogels, listing gene expression trends over time (Fig. S3E, Supporting Information). Results indicated that chondrocytes in Col-HZ dynamic hydrogels exhibited higher differentiation levels compared to GelMA, further corroborating that Col-HZ dynamic hydrogels impact the Wnt/β-catenin signaling pathway, thereby enhancing chondrocyte matrix deposition.

The Wnt/β-catenin signaling pathway is a canonical Wnt pathway that regulates β-catenin stability and nuclear localization [50,51]. This pathway transmits extracellular signals into the cell, playing crucial roles in cell proliferation, differentiation, and tissue homeostasis. Our study indicates that Col-HZ dynamic hydrogels, constructed through reversible hydrazone bonds, significantly enhance chondrocyte matrix deposition by modulating the Wnt/β-catenin signaling pathway. When chondrocytes interact with the Col-HZ dynamic hydrogel, the dynamic network skeleton of the hydrogel responds to cellular movement. The cell membrane transmits these external dynamic mechanical signals to the interior of the cells through the Wnt/β-catenin signaling pathway. This interaction triggers the upregulation of genes involved in cartilage matrix deposition, facilitating effective cartilage regeneration and repair. This mechanism underscores the importance of dynamic hydrogels in enhancing chondrocyte activity and supporting cartilage tissue engineering. Moreover, prior studies suggest that dynamic hydrogels enhance chondrocyte matrix synthesis by activating key mechanotransduction molecules such as YAP/TAZ and PIEZO1 [36].

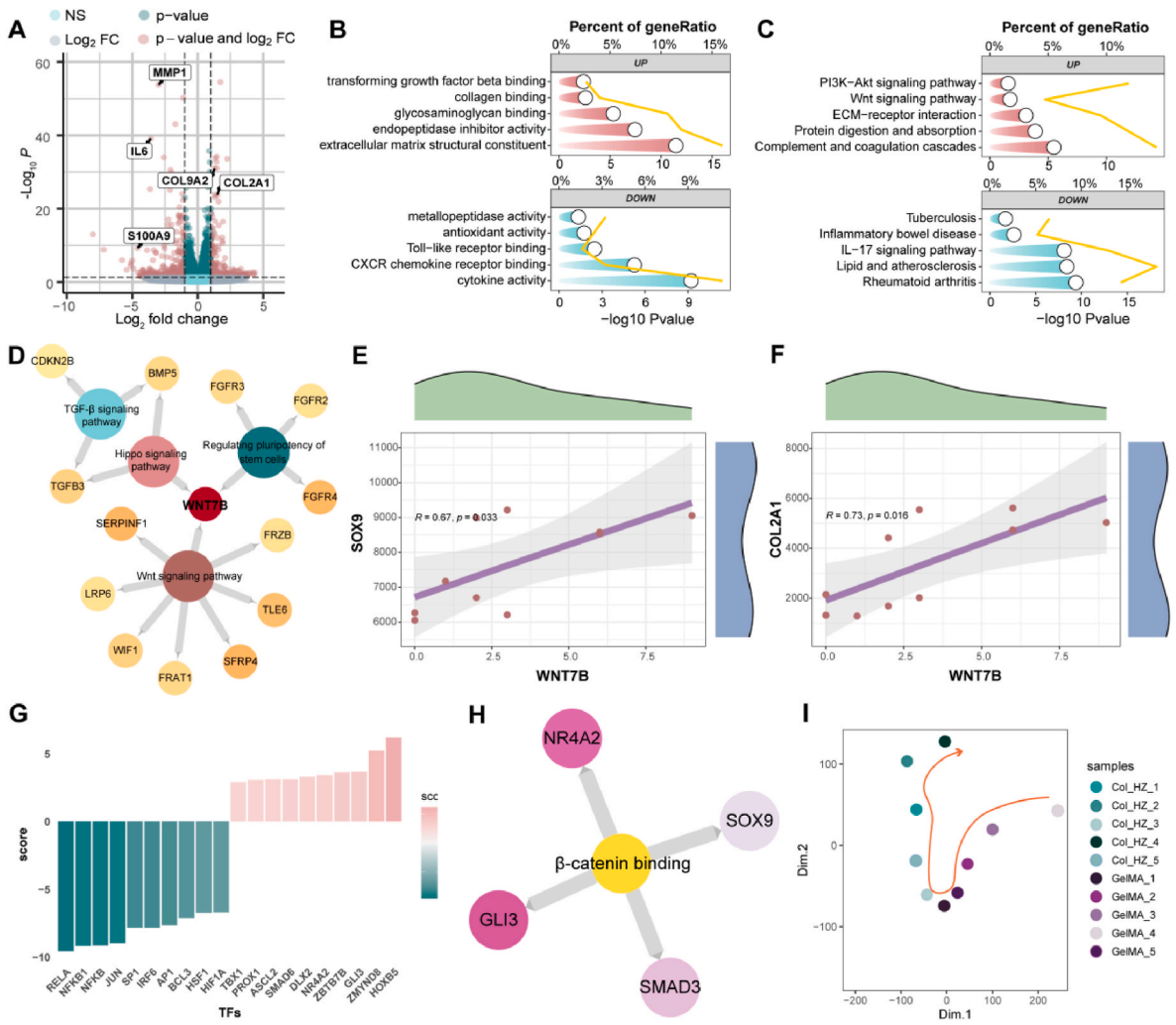


Fig. 4. Mechanism of Promoting Matrix Deposition with Dynamic Col-HZ Hydrogel. (A) A volcano plot illustrates the differentially expressed genes (DEGs) between Col-HZ dynamic hydrogels and GelMA static hydrogels, highlighting significant genes. (B) GO enrichment analysis of the DEGs from both groups. (C) KEGG enrichment analysis of the DEGs from both groups. (D) Network interaction analysis of the signaling pathways up-regulated in Col-HZ dynamic hydrogels. (E, F) GSEA confirms a positive correlation between WNT7B and the key chondrogenic factors SOX9 and COL9A2. (G) Transcription factor activity analysis based on differential gene expression, listing significantly up-regulated and down-regulated key transcription factors in Col-HZ dynamic hydrogels compared to GelMA static hydrogels. (H) GO enrichment analysis of the significantly up-regulated transcription factors. (I) Predicted differentiation direction analysis based on gene expression variation for all groups in Col-HZ dynamic hydrogels and GelMA static hydrogels.

2.5. Mechanism of TD-198946 in enhancing Chondrocyte matrix deposition

TD-198946 is a small molecule compound shown to promote cartilage differentiation and repair [18,52]. Through the Schiff base reactionreactions between TD-198946 and Col-CHO, it can be incorporated into the Col-HZ hydrogel framework. As demonstrated in Fig. 3, the TD-198946-loaded Col-HZ-TD hydrogel further enhances chondrocyte matrix deposition. To explore the underlying mechanisms, we performed RNA sequencing (RNA-Seq) analysis.

Gene expression profiles of the Col-HZ and Col-HZ-TD hydrogel groups were clustered, revealing significant differences in gene expression (Fig. S4A, Supporting Information). Differential gene analysis identified key genes (Fig. 5A). GO and KEGG enrichment analyses of these differential genes were subsequently performed (Fig. 5B and C). GO analysis indicated that, compared to the Col-HZ hydrogel, the Col-HZ-TD hydrogel further promotes ECM deposition and regeneration processes. KEGG analysis showed that the Col-HZ-TD hydrogel downregulates cellular senescence pathways while upregulating Wnt signaling and ECM deposition-related pathways. Network interaction analysis highlighted the critical regulatory role of the Wnt signaling pathway in controlling multiple pathways related to cartilage matrix deposition and senescence (Fig. 5D).

GSEA-GO analysis indicated that the Col-HZ-TD hydrogel promotes the biological process of ECM organization (Fig. 5E). The ECM provides a three-dimensional environment that regulates chondrocyte proliferation, differentiation, and metabolic activities, while also imparting elasticity and compressive strength to cartilage tissue, which is crucial for enhancing cartilage regeneration and repair. Additionally, GSEA-KEGG analysis revealed that the Col-HZ-TD hydrogel further upregulates the Wnt signaling pathway (Fig. 5F).

We also performed transcription factor activity analysis, which highlighted both upregulated and downregulated transcription factors in the Col-HZ-TD group (Fig. 5G). Notably, the transcription factor CTNNB1, encoding β-catenin—a key component of the Wnt signaling pathway—was significantly upregulated. Moreover, CTNNB1 regulates several targets directly associated with cartilage regeneration, including TGFB2, IGF1, and BMP2. Molecular docking simulations predicted that TD-198946 enhances the transcriptional regulatory effects on these cartilage regeneration-related genes by binding to β-catenin, a key

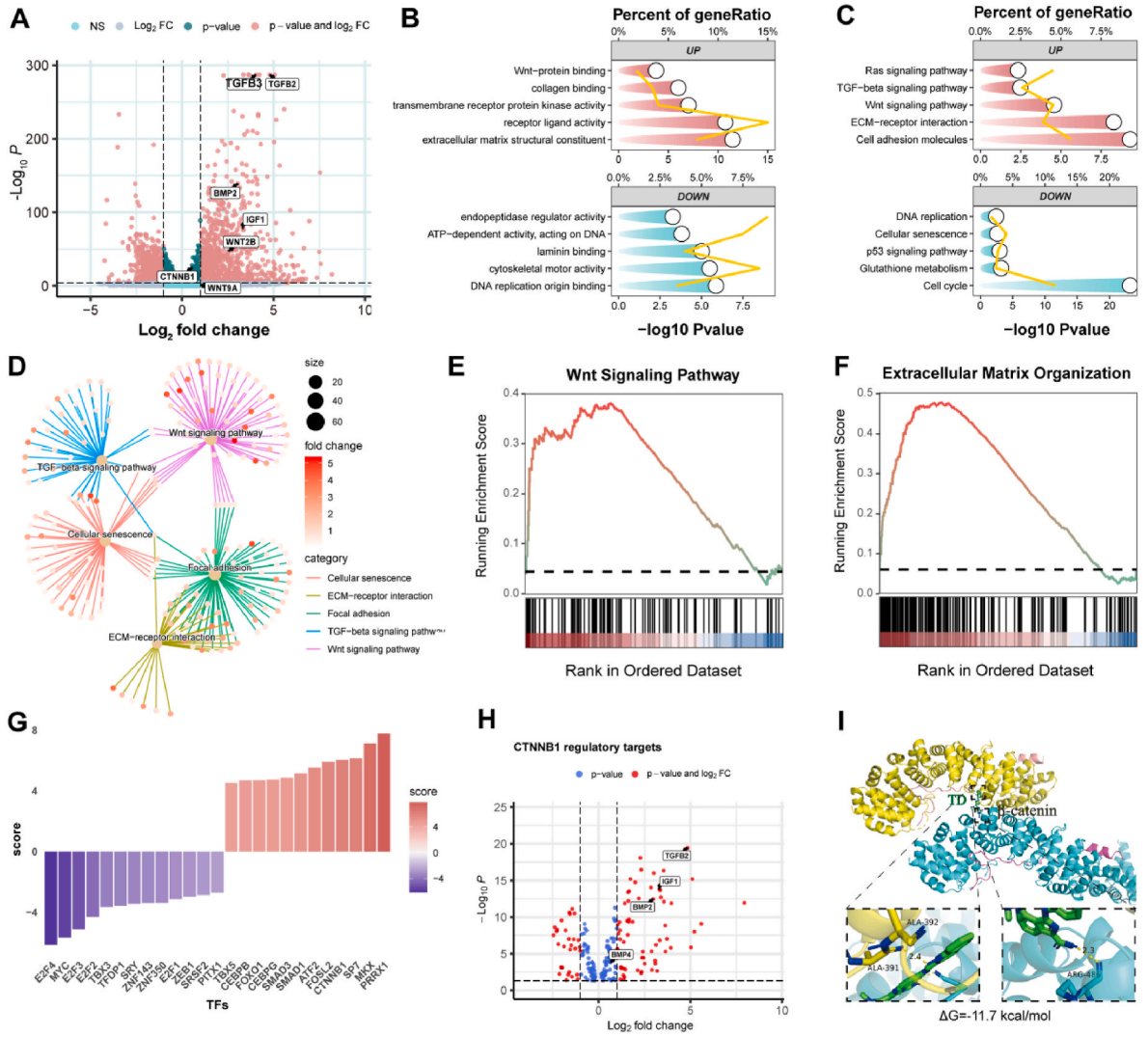


Fig. 5. The mechanism of promoting matrix deposition with TD-198946. (A) Differential gene analysis comparing the Col-HZ and Col-HZ-TD groups, highlighting significant differential genes. (B, C) GO and KEGG enrichment analyses of the differential genes. (D) Network interaction analysis of signaling pathways. (E) GSEA-GO analysis indicating biological processes favoring ECM organization. (F) GSEA-KEGG analysis showing upregulation of the Wnt signaling pathway in the Col-HZ-TD hydrogel.

transcription factor in the Wnt pathway [53,54].

While previous studies have confirmed TD-198946's role in promoting cartilage formation and repair, primarily through its mediation of stem cell chondrogenic differentiation, our research provides novel insights. We demonstrate that in primary chondrocytes, TD-198946 enhances ECM deposition and inhibits chondrocyte senescence by regulating the Wnt/β-catenin signaling pathway. This finding deepens our understanding of TD-198946's role in cartilage repair and regeneration.

Thus, both the dynamic network structure of the Col-HZ hydrogel and the dynamic binding of TD-198946 synergistically enhance cartilage matrix deposition via the mediation of the Wnt/β-catenin signaling pathway, underscoring its significant role in transmitting mechanical and chemical signals.

2.6. In vivo experiments demonstrate the effect of Dynamic Col-HZ Hydrogel on cartilage matrix deposition

To validate the in vivo effects of Col-HZ hydrogels on cartilage matrix deposition, subcutaneous implantation experiments were performed in nude mice. As depicted in Fig. 6A, healthy nude mice (6–8 weeks old) were anesthetized, and a small incision was made in the dorsal skin. Ear-shaped hydrogels encapsulating chondrocytes were implanted subcutaneously into the dorsal region using tweezers. After 5 weeks, the mice were sacrificed, and the implanted hydrogels were harvested and analyzed through histological staining.

Initial HE staining results of the heart, liver, and kidneys showed no significant morphological changes in tissue structure, suggesting that the dynamic Col-HZ hydrogels exhibited good biocompatibility in vivo (Fig. S5, Supporting Information). Next, the harvested hydrogel samples were stained and observed (Fig. 6B). After 5 weeks, all hydrogel groups provided structural support for the chondrocytes. HE staining revealed that cells in the dynamic hydrogels were more densely clustered, forming chondrocyte aggregates, while cells in the static GelMA hydrogel were more dispersed. Alcian blue staining showed deeper blue coloration in both the Col-HZ and Col-HZ-TD dynamic hydrogel groups, indicative of greater cartilage matrix deposition. Collagen II immunohistochemistry further demonstrated larger positive staining areas in the Col-HZ-TD hydrogel group, with Col-HZ outperforming the static GelMA hydrogel. These findings were confirmed through quantitative analysis (Fig. 6C and D).

In summary, the Col-HZ-TD dynamic hydrogel group exhibited enhanced cartilage matrix deposition in vivo, although some loss of the initial ear shape was observed. In contrast, the static GelMA hydrogel

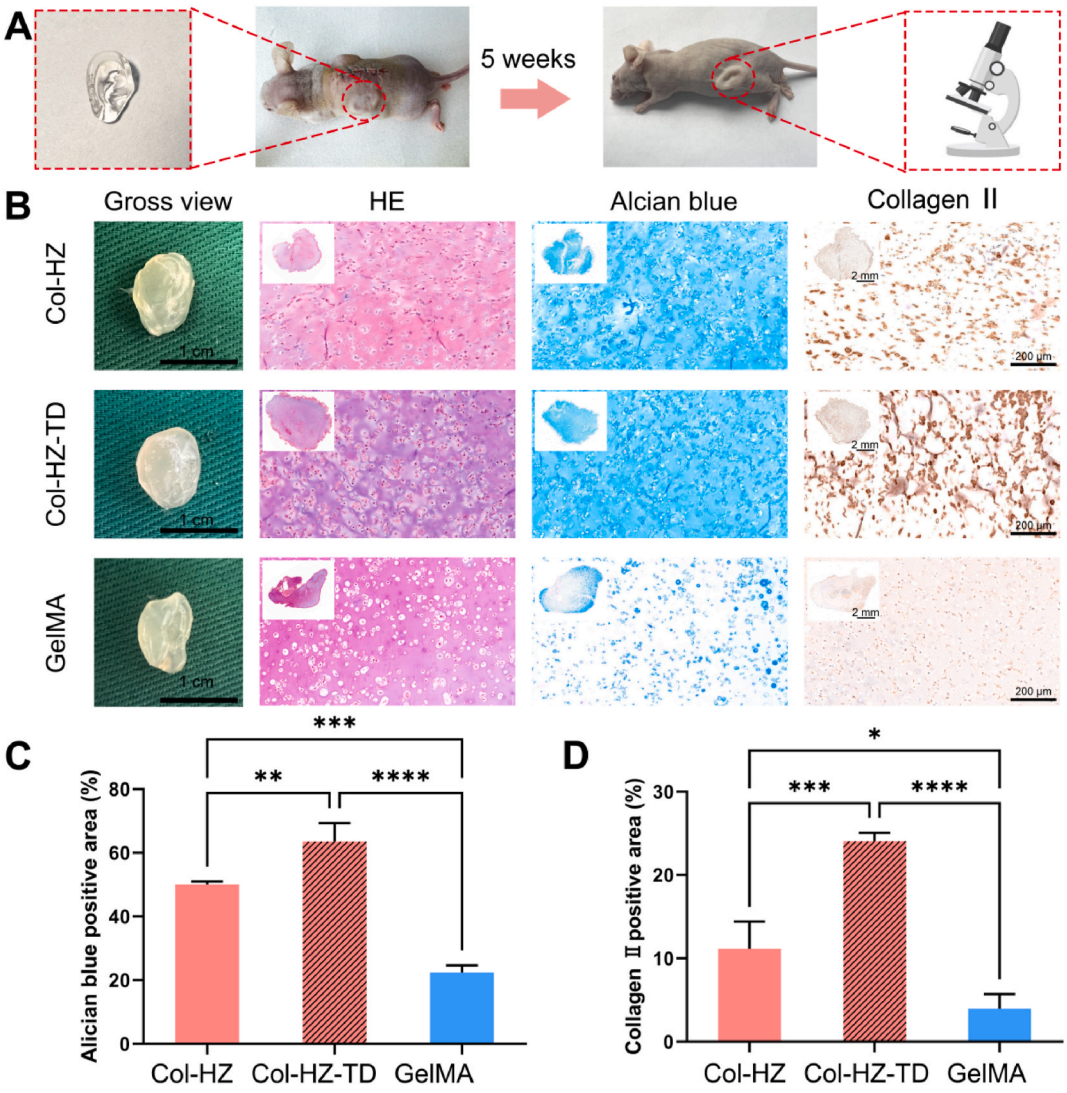


Fig. 6. In Vivo Cartilage Regeneration with Dynamic Col-HZ-TD Hydrogel. (A) Schematic of the in vivo experiment. (B) After 5 weeks of subcutaneous implantation in nude mice, the hydrogels were harvested for observation and staining analysis. (C) Quantification of Alcian blue staining. (D) Quantification of Collagen II immunohistochemical staining.

group better preserved the ear shape but showed limited cartilage matrix formation. This suggests that while the dynamic properties of hydrogels can improve cartilage repair by promoting matrix deposition, maintaining the shape may require additional structural support, which will be explored in future studies.

3. Conclusion

Traditional hydrogels, with their limited network space and low bioactivity, fall short in supporting effective cartilage matrix deposition and repair. To address these limitations, we developed a Col-HZ dynamic hydrogel with an adaptive network formed by hydrazone bonds between collagen and PEG. This simple synthesis process creates a dynamic hydrogel that significantly enhances chondrocyte migration and matrix deposition compared to static hydrogels. By incorporating TD-198946 through Schiff base interactions, we further enhanced matrix deposition and reduced inflammation. Transcriptomic and bioinformatics analyses revealed that both the dynamic network and TD-198946 promote cartilage formation by activating the Wnt/β-catenin pathway. In vivo experiments confirmed the superior chondrogenic effects of the hydrogel, underscoring its potential for cartilage repair in diverse tissues, including the ear, nose, trachea, and joints.

4. Experimental section

4.1. Materials

Recombinant human collagen type I was purchased from Trautec (China). All PEGs were obtained from SINOPEG (China), and GelMA was sourced from EFL (China). Other materials were purchased from Sigma-Aldrich unless otherwise specified.

4.2. Fabrication of Col-HZ Hydrogel

Col and SC-PEG-CHO powder were dissolved in 0.1 M phosphate buffer (PB) to the desired concentration. After complete dissolution, the solution was transferred to a shaking chamber at 37 °C. After sufficient reaction, Col-CHO was synthesized by grafting aldehyde groups onto collagen. Next, 8-arm-PEG-Hy powder was dissolved in 0.01 M phosphate-buffered saline (PBS) with 4-Amino-DL-Phenylalanine as a catalyst. Equal volumes of Col-CHO and PEG-HZ solutions were mixed and vortexed immediately. The resulting precursor solution was then injected into various molds or culture plates prior to gelation. For cell and animal experiments, all solutions were filtered using 0.2 μm syringe filters (PALL, USA).

4.3. Characterization of Col-HZ Hydrogel

Fourier transform infrared (FTIR) spectroscopy was used to study the formation of the Col-HZ hydrogel. The Col-CHO, 8-arm-PEG-Hy, and Col-HZ hydrogels were lyophilized and ground into powder. After potassium bromide tableting, FTIR spectra were obtained using a TENSOR-27 spectrometer (Bruker, Germany).

Gelation time was assessed by mixing the Col-CHO and 8-arm-PEG-Hy solutions in a centrifuge tube, followed by gentle inversion until no flow was observed, marking the gelation point.

To examine the internal porous structure of the Col-HZ hydrogel, the gel was freeze-dried after gelation. The morphology was then observed using a scanning electron microscope (SEM, S-3400, Hitachi) at a voltage of 10 kV.

The mechanical properties of the hydrogel were assessed using a rotary rheometer (Thermo Scientific Haake MARS III). The hydrogel precursor solution was injected into a mold to form discs with a diameter of 20 mm and a height of 1 mm. The linear viscoelastic region was first defined, and then an oscillation frequency ranging from 0.1 Hz to 10 Hz was applied. The storage modulus (G′) and loss modulus (G″) were measured (n = 5). Stress relaxation properties were evaluated by applying 10 % strain in rotational testing (n = 3).

To evaluate the degradation process of the hydrogels, a swelling experiment was performed. Specifically, the hydrogels were molded into cylindrical shapes (R = 10 mm, H = 5 mm) and individually immersed in 10 mL of PBS. The hydrogels were weighed at predetermined time intervals to monitor the changes in their mass over time.

4.4. Cell culture

Auricular cartilage specimens were collected from microtia patients (aged 9–12 years) attending the Eye & ENT Hospital of Fudan University (Shanghai, P.R. China). Informed consent was obtained from all participants. Fibrous tissue was carefully removed from each cartilage specimen to isolate chondrocytes. The cartilage specimens were minced and digested with 0.15 % collagenase (Nordmark, Uetersen, Germany) to isolate chondrocytes. After quantification, primary chondrocytes were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Thermo Fisher Scientific, MA, USA) supplemented with 10 % fetal bovine serum (FBS, Hyclone Laboratories, Victoria, Australia). Chondrocytes at passages 3–4 were used for subsequent experiments.

4.5. Chondrocyte movement in hydrogels

The gel-forming components were prepared as described above. The hydrogel precursor solution, containing chondrocytes, was injected into a 48-well plate. After gelation, the chondrocytes were uniformly dispersed throughout the hydrogel. Following digestion with trypsin, the cultured chondrocytes were counted. The chondrocytes were centrifuged and resuspended in the hydrogel precursor solution at a concentration of 1×10^6 cells/mL. The hydrogel precursor solution with chondrocytes was then injected into a 48-well plate, forming a hydrogel with uniformly distributed chondrocytes upon gelation. The plate was inverted, and a straight line (red line) was drawn at the center of each well using a ruler and marker. The hydrogel on the right side of the line was removed with the flat end of a spatula, and the resultant space was filled with a cell-free hydrogel precursor solution. Live/dead staining was performed on days 1, 7, and 14 to assess cell migration (blue line). At selected time points, cells were washed with PBS and stained. Cell images were captured using a confocal laser scanning microscope (CLSM, A1, Nikon, Japan). The images were processed using ImageJ to calculate the migration distance, based on more than five images per group. Additionally, cell morphology was analyzed using Phalloidin-FITC and DAPI labeling. Cells were imaged using CLSM (SP8, Leica, Japan).

4.6. Chondrocyte matrix deposition in hydrogels

After culturing the chondrocytes in the hydrogel for 14 days, they were fixed with paraformaldehyde. Hematoxylin and eosin (H&E) staining, Alcian blue staining, and immunohistochemistry for type II collagen were performed following established protocols in our laboratory.

4.7. RNA isolation and sequencing

After 7 days of culture, cells were lysed for quantitative real-time polymerase chain reaction (qRT-PCR) analysis according to the manufacturer's instructions. Highly purified primers were synthesized commercially and are listed in Table S1 cDNA quantification was performed using a CFX96 Touch PCR detection system (Bio-Rad, USA). The relative expression levels of each gene (fold change) were calculated using the comparative Ct (2-△△Ct) method. All experiments were conducted in triplicate to obtain average data (n = 3).

4.8. Transcriptome sequencing

Chondrocytes encapsulated in hydrogels after 14 days of culture were lysed, and gene expression results were obtained using the Illuminate NovaSeq 6000 sequencing platform. Differential expression analysis and functional enrichment analysis were conducted using multiple bioinformatics tools.

4.9. Subcutaneous implantation

Male nude mice (6–8 weeks old, GemPharmatech, Nanjing, China) were anesthetized using isoflurane (2 % carried by oxygen) and maintained under anesthesia with a nose cone containing 1.5–2% isoflurane. The hydrogel precursor solution containing chondrocytes was injected into a mold shaped like an auricle. The auricle-shaped hydrogel was then implanted subcutaneously in the dorsal region of the nude mice. After 5 weeks, the hydrogels were harvested and processed for histochemical and immunofluorescence staining analyses. All animal experiments were approved by the Institutional Animal Care and Use Committee of Fudan University.

4.10. Statistical analysis

Data are expressed as mean ± standard deviation (SD) (n ≥ 3). All statistical analyses were performed using one-way ANOVA with Tukey's multiple comparisons test and two-tailed Student's t-test. Statistical significance between two datasets was indicated as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

CRediT authorship contribution statement

Honglei Wang: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Xu Wu:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lili Chen:** Software, Resources, Investigation. **Hua Tong:** Visualization, Software, Project administration. **Xuerui Hu:** Resources, Investigation. **Aijuan He:** Writing – review & editing, Supervision. **Chenlong Li:** Supervision, Resources. **Xudong Guo:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Yaoyao Fu:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Tianyu Zhang:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

Honglei Wang and Xu Wu contributed equally to this work.

This research was supported by Postdoctoral Fellowship Program of CPSF under Grant Number GZC20230503, Shanghai Science and Technology Development Foundation Research and Innovation Special Fund (SKH2023D1), Shanghai Science and Technology Innovation Action Plan (23Y21900200, 21DZ2200700).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtbio.2025.101623.

Data availability

Data will be made available on request.

References

[1] Y. Krishnan, A.J. Grodzinsky, Cartilage diseases, Matrix Biol. 71 (2018) 51–69.
[2] Y. Li, Y. Xiao, C. Liu, The horizon of materiobiology: a perspective on material-guided cell behaviors and tissue engineering, Chem. Rev. 117 (5) (2017) 4376–4421.
[3] L. Yang, S. Pijuan-Galito, H.S. Rho, A.S. Vasilevich, A.D. Eren, L. Ge, P. Habibovic, M.R. Alexander, J. de Boer, A. Carlier, P. van Rijn, Q. Zhou, High-throughput methods in the discovery and study of biomaterials and materiobiology, Chem. Rev. 121 (8) (2021) 4561–4677.
[4] P.L. Zhang, Q.Y. Wang, J. Chen, Z. Ci, W. Zhang, Y. Liu, X.Y. Wang, G.D. Zhou, Chondrogenic medium in combination with a c-Jun N-terminal kinase inhibitor mediates engineered cartilage regeneration by regulating matrix metabolism and cell proliferation, Regener, Biomater 10 (2023) rbad079.
[5] M.H. Norahan, S.C. Pedroza-Gonz, M.G. Sanchez-Salazar, M.M. Alvarez, G.T. d. Santiago, Structural and biological engineering of 3D hydrogels for wound healing, Bioact. Mater. 24 (2023) 197–235.
[6] T. Ho, C. Chang, H. Chan, T. Chung, C. Shu, K. Chuang, T. Duh, M. Yang, Y. Tyan, Hydrogels: properties and applications in biomedicine, Molecules 27 (9) (2022) 2902.
[7] L. Guo, X. Liu, Y. Wang, J. Yi, J. Li, Y. Xu, K. Cai, W. Dai, Q. Feng, B. Tao, Enhancing long-segmental tracheal restoration: a self-repairing hydrogel loaded with chondrocytokines for sutureless anastomosis and cartilage regeneration, Mater. Today Bio 28 (2024) 101208.
[8] H.L. Wang, R.Z. Huang, L. Bai, Y.X. Cai, M. Lei, C.Y. Bao, S.L. Lin, S.Z. Ji, C.S. Liu, X. Qu, Extracellular matrix-mimetic immunomodulatory hydrogel for accelerating wound healing, Adv. Healthcare Mater. 12 (27) (2023) 2301264.
[9] A.M. Rosales, K.S. Anseth, The design of reversible hydrogels to capture extracellular matrix dynamics, Nat. Rev. Mater. 1 (2) (2016) 1–15.
[10] Z. Tong, L. Jin, J.M. Oliveira, R.L. Reis, Q. Zhong, Z. Mao, C. Gao, Adaptable hydrogel with reversible linkages for regenerative medicine: dynamic mechanical microenvironment for cells, Bioact, Mater 6 (5) (2021) 1375–1387.
[11] O. Chaudhuri, L. Gu, D. Klumpers, M. Darnell, S.A. Bencherif, J.C. Weaver, N. Huebsch, H.-p. Lee, E. Lippens, G.N. Duda, D.J. Mooney, Hydrogels with tunable stress relaxation regulate stem cell fate and activity, Nat. Mater. 15 (3) (2016) 326–334.
[12] Z. Li, B. Yang, Z. Yang, X. Xie, Z. Guo, J. Zhao, R. Wang, H. Fu, P. Zhao, X. Zhao, G. Chen, G. Li, F. Wei, L. Bian, Supramolecular hydrogel with ultra-rapid cell-mediated network adaptation for enhancing cellular metabolic energetics and tissue regeneration, Adv. Mater. 36 (15) (2024) 2307176.
[13] S. Uman, A. Dhand, J.A. Burdick, Recent advances in shear-thinning and self-healing hydrogels for biomedical applications, J. Appl. Polym. Sci. 137 (25) (2020) 48668.
[14] A. Bandzerewicz, A. Gadomska-Gajadhur, Into the tissues: extracellular matrix and its artificial substitutes: cell signalling mechanisms, Cells 11 (5) (2022) 914.
[15] W. Zhang, Y. Liu, H. Zhang, Extracellular matrix: an important regulator of cell functions and skeletal muscle development, Cell Biosci. 11 (1) (2021) 65.
[16] M.P. Murphy, L.S. Koepke, M.T. Lopez, X. Tong, T.H. Ambrosi, G.S. Gulati, O. Marecic, Y. Wang, R.C. Ransom, M.Y. Hoover, H. Steininger, L. Zhao, M. P. Walkiewicz, N. Quarto, B. Levi, D.C. Wan, I.L. Weissman, S.B. Goodman, F. Yang, M.T. Longaker, C.K.F. Chan, Articular cartilage regeneration by activated skeletal stem cells, Nat. Med. 26 (10) (2020) 1583–1592.
[17] K. Johnson, S. Zhu, M.S. Tremblay, J.N. Payette, J. Wang, L.C. Bouchez, S. Meeusen, A. Althage, C.Y. Cho, X. Wu, P.G. Schultz, A stem cell-based approach to cartilage repair, Science 336 (6082) (2012) 717–721.
[18] R. Chijimatsu, F. Yano, T. Saito, M. Kobayashi, S. Hamamoto, T. Kaito, J. Kushioka, D.A. Hart, U.-i. Chung, S. Tanaka, H. Yoshikawa, N. Nakamura, Effect of the small compound TD-198946 on glycosaminoglycan synthesis and transforming growth factor β3-associated chondrogenesis of human synovium-derived stem cells in vitro, J. Tissue Eng. Regener 13 (3) (2019) 446–458.
[19] F. Yano, H. Hojo, S. Ohba, A. Fukai, Y. Hosaka, T. Ikeda, T. Saito, M. Hirata, H. Chikuda, T. Takato, H. Kawaguchi, U.-i. Chung, A novel disease-modifying osteoarthritis drug candidate targeting Runx1, Ann. Rheum. Dis. 72 (5) (2013) 748–753.
[20] X. Guo, X.-F. Wang, Signaling cross-talk between TGF-β/BMP and other pathways, Cell Res. 19 (1) (2009) 71–88.
[21] T. Li, B. Liu, K. Chen, Y. Lou, Y. Jiang, D. Zhang, Small molecule compounds promote the proliferation of chondrocytes and chondrogenic differentiation of stem cells in cartilage tissue engineering, Biomed. Pharmacother. 131 (2020) 110652.
[22] H. Tan, H. Wang, Y. Chai, Y. Yu, H. Hong, F. Yang, X. Qu, C. Liu, Engineering a favourable osteogenic microenvironment by heparin mediated hybrid coating assembly and rhBMP-2 loading, RSC Adv. 7 (19) (2017) 11439–11447.
[23] A.M. Vargason, A.C. Anselmo, S. Mitragotri, The evolution of commercial drug delivery technologies, Nat. Biomed. Eng. 5 (9) (2021) 951–967.
[24] W. He, J. Bai, X. Chen, D. Suo, S. Wang, Q. Guo, W. Yin, D. Geng, M. Wang, G. Pan, X. Zhao, B. Li, Reversible dougong structured receptor-ligand recognition for building dynamic extracellular matrix mimics, Proc. Natl. Acad. Sci. USA 119 (8) (2022) e2117221119.
[25] I.N. Amirrah, Y. Lokanathan, I. Zulkiflee, M.F.M.R. Wee, A. Motta, M.B. Fauzi, A comprehensive review on collagen type I development of biomaterials for tissue engineering: from biosynthesis to bioscaffold, Biomedicines 10 (9) (2022) 2307.
[26] M.R. Buckley, E.B. Evans, P.E. Matuszewski, Y.-L. Chen, L.N. Satchel, D.M. Elliott, L.J. Soslowsky, G.R. Dodge, Distributions of types I, II and III collagen by region in the human supraspinatus tendon, Connect. Tissue Res. 54 (6) (2013) 374–379.
[27] R. Huang, X. Niu, X. Li, X. Li, Applications of type I and II collagen in osteochondral tissue engineering: respective features and future perspectives, Medicine in Novel Technology and Devices 24 (2024) 100328.
[28] M. Kino-Oka, S. Yashiki, Y. Ota, Y. Mushiaki, K. Sugawara, T. Yamamoto, T. Takezawa, M. Taya, Subculture of chondrocytes on a collagen type I-coated substrate with suppressed cellular dedifferentiation, Tissue Eng. 11 (3–4) (2005) 597–608.
[29] C. Kilmer, C.M. Battistoni, A. Cox, G.J. Breur, A. Panitch, J.C. Liu, Collagen type I and II blend hydrogel with autologous mesenchymal stem cells as a scaffold for articular cartilage defect repair, ACS Biomater. Sci. Eng. 6 (6) (2020) 3464–3476.
[30] V. Irawan, T.-C. Sung, A. Higuchi, T. Ikoma, Collagen scaffolds in cartilage tissue engineering and relevant approaches for future development, Tissue Eng. Regen. Med. 15 (6) (2018) 673–697.
[31] A. Brown, H. He, E. Trumper, J. Valdez, P. Hammond, L.G. Griffith, Engineering PEG-based hydrogels to foster efficient endothelial network formation in free-swelling and confined microenvironments, Biomaterials 243 (2020) 119921.
[32] E.T. Kool, D.-H. Park, P. Crisalli, Fast hydrazone reactants: electronic and acid/base effects strongly influence rate at biological pH, J. Am. Chem. Soc. 135 (47) (2013) 17663–17666.
[33] M.V. Ghica, M. Hirjau, D. Lupuleasa, C.-E. Dinu-Pirvu, Flow and thixotropic parameters for rheological characterization of hydrogels, Molecules 21 (6) (2016) 786.
[34] G.D. O'Connell, A.R. Tan, V. Cui, J.C. Bulinski, J.L. Cook, M. Attur, S.B. Abramson, G.A. Ateshian, C.T. Hung, Human chondrocyte migration behaviour to guide the development of engineered cartilage, J. Tissue Eng. Regener. 11 (3) (2017) 877–886.
[35] F. Qu, F. Guilak, R.L. Mauck, Cell migration: implications for repair and regeneration in joint disease, Nat. Rev. Rheumatol. 15 (3) (2019) 167–179.
[36] T. Hodgkinson, D.C. Kelly, C.M. Curtin, F.J. O'Brien, Mechanosignalling in cartilage: an emerging target for the treatment of osteoarthritis, Nat. Rev. Rheumatol. 18 (2) (2022) 67–84.
[37] W. Xie, X. Wei, H. Kang, H. Jiang, Z. Chu, Y. Lin, Y. Hou, Q. Wei, Static and dynamic: evolving biomaterial mechanical properties to control cellular mechanotransduction, Adv. Sci. 10 (9) (2023) 2204594.
[38] F.J. Vernerey, S.L. Sridhar, A. Muralidharan, S.J. Bryant, Mechanics of 3D cell-hydrogel interactions: experiments, models, and mechanisms, Chem. Rev. 121 (18) (2021) 11085–11148.
[39] A.C. Hall, The role of chondrocyte morphology and volume in controlling PhenotypeImplications for osteoarthritis, cartilage repair, and cartilage engineering, Curr. Rheumatol. Rep. 21 (8) (2019) 1–13.
[40] A. Karim, A.C. Hall, Chondrocyte morphology in stiff and soft agarose gels and the influence of fetal calf serum, J. Cell. Physiol. 232 (5) (2017) 1041–1052.
[41] M. Wang, Y. Wu, G. Li, Q. Lin, W. Zhang, H. Liu, J. Su, Articular cartilage repair biomaterials: strategies and applications, Mater. Today Bio 24 (2024) 100948.
[42] Z. Peng, H. Sun, V. Bunpetch, Y. Koh, Y. Wen, D. Wu, H. Ouyang, The regulation of cartilage extracellular matrix homeostasis in joint cartilage degeneration and regeneration, Biomaterials 268 (2021) 120555.
[43] Y. Gao, S. Liu, J. Huang, W. Guo, J. Chen, L. Zhang, B. Zhao, J. Peng, A. Wang, Y. Wang, W. Xu, S. Lu, M. Yuan, Q. Guo, The ECM-cell interaction of cartilage extracellular matrix on chondrocytes, BioMed Res. Int. 2014 (1) (2014) 648459.
[44] Z. Gu, J. Wang, Y. Fu, H. Pan, H. He, Q. Gan, C. Liu, Smart biomaterials for articular cartilage repair and regeneration, Adv. Funct. Mater. 33 (10) (2023) 2212561.
[45] Y. Hu, C. Lyu, L. Teng, A. Wu, Z. Zhu, Y. He, J. Lu, Glycopolypeptide hydrogels with adjustable enzyme-triggered degradation: a novel proteoglycans analogue to repair articular-cartilage defects, Mater. Today Bio 20 (2023) 100659.
[46] A. Yosef, O. Kossover, I. Mironi-Harpaz, A. Mauretti, S. Melino, J. Mizrahi, D. Seliktar, Fibrinogen-based hydrogel modulus and ligand density effects on cell

morphogenesis in two-dimensional and three-dimensional cell cultures, Adv. Healthcare Mater. 8 (13) (2019) 1801436.
[47] Y. Ling, W. Zhang, P. Wang, W. Xie, W. Yang, D.-A. Wang, C. Fan, Three-dimensional (3D) hydrogel serves as a platform to identify potential markers of chondrocyte dedifferentiation by combining RNA sequencing, Bioact. Mater. 6 (9) (2021) 2914–2926.
[48] J.L. Pablos, D. Lozano, M. Manzano, M. Vallet-Regi, Regenerative medicine: hydrogels and mesoporous silica nanoparticles, Materials today. Bio 29 (2024) 101342, 101342.
[49] Y. Ma, T. Han, Q. Yang, J. Wang, B. Feng, Y. Jia, Z. Wei, F. Xu, Viscoelastic cell microenvironment: hydrogel-based strategy for recapitulating dynamic ECM mechanics, Adv. Funct. Mater. 31 (24) (2021) 2100848.
[50] J. Liu, Q. Xiao, J. Xiao, C. Niu, Y. Li, X. Zhang, Z. Zhou, G. Shu, G. Yin, Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities, Signal Transduct. Tar. 7 (1) (2022) 3.
[51] G.G. Tortelote, R.R. Reis, F.d.A. Mendes, J.G. Abreu, Complexity of the Wnt/β-catenin pathway: searching for an activation model, Cell. Signal. 40 (2017) 30–43.
[52] M. Kobayashi, R. Chijimatsu, D.A. Hart, S. Hamamoto, G. Jacob, F. Yano, T. Saito, K. Shimomura, W. Ando, U.-i. Chung, S. Tanaka, H. Yoshikawa, N. Nakamura, Evidence that TD-198946 enhances the chondrogenic potential of human synovium-derived stem cells through the NOTCH3 signaling pathway, J. Tissue Eng. Regener. 15 (2) (2021) 103–115.
[53] L.H.S. Santos, R.S. Ferreira, E.R. Caffarena, Integrating Molecular Docking and Molecular Dynamics Simulations, Springer, 2019.
[54] A. Vidal-Limon, J.E. Aguilar-Toala, A.M. Liceaga, Integration of molecular docking analysis and molecular dynamics simulations for studying food proteins and bioactive peptides, J. Agric. Food Chem. 70 (4) (2022) 934–943.