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REVIEW

Advanced injectable hydrogels for bone tissue regeneration

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Abstract

Diseases or defects of the skeleton are hazardous because of their specificity and intricacy. Bone tissue engineering has become an important area of research that offers promising new tools for making biomimetic hydrogels that can be used to treat bone diseases. New hydrogels with a distinctive 3D network structure, high water content, and functional capabilities are ranked among the most promising candidates for bone tissue engineering. This makes them helpful in treating cartilage injury, skull deformity, and arthritis. This review will briefly introduce the variety of biocompatible functional hydrogels used in cell culture and bone tissue regeneration. Many gel design concepts, such as crosslinking procedures, controlled release properties, and alternative bionic methodology, were stressed regarding injectable hydrogels to form bone tissue. Hydrogels manufactured from biocompatible materials are a promising option for minimally invasive surgery because of their adaptable physicochemical qualities, ability to fill irregularly shaped defect sites, and ability to grow hormones or release drugs in response to external stimuli. Also included in this overview is a quick rundown of the more practical designs employed in treating bone disorders. Essential details on injectable hydrogel scaffolds for bone tissue regeneration are described in this article.

Keywords Bone defect · Injectable hydrogels · Biopolymers · Tissue engineering

Abbreviations

BMP Bone morphogenetic protein
CS Chondroitin sulfate
CSE Corn silk extract
ECM Extracellular matrix
FGF Fibroblast growth factor
HA Hyaluronic acid
IGF Insulin-like growth factor
NPs Nanoparticles
ROS Reactive oxygen species
PLA Polycaprolactone
TGF Transforming growth factor

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Introduction

Bone tissue, as a dynamic load-bearing tissue, serves a variety of critical physiological activities for the body, such as maintaining body shape, protecting bone marrow or organs, and aiding in the body's detoxification processes (Ahmadi, Pilehvar, Zarghami, & Abri, 2021). Fractures, bone abnormalities, and osteoarthritis, among other bone-related diseases brought on by injury, infection, or age-related illnesses, continue to substantially impact patients' quality of life (Stolzing, Jones, Mcgonagle, & Scutt, 2008). The hematoma, hematoma organization, callus, and remodeling phases are the typical four phases that occur during the healing of fractures or injuries. Immune cells like neutrophils and monocytes are drawn in during the first stage, characterized by high-level inflammation that results in the formation of hematomas. TNF and IL-1 played essential roles during this time. Under the control of the anti-inflammatory factors

IL-10 and IL-4, inflammation and immune response rapidly decrease in the second phase. At the start of the callus phase, activated TGF signaling attracts mesenchymal stem cells (MSCs), promotes chondrogenic differentiation, and hastens angiogenesis. The newly formed cartilage is then digested by epithelial cells, triggering osteogenesis and a rapid rate of bone turnover. Osteoclasts and related cell factors like matrix metalloproteinases (MMPs) break down the disordered woven bone during the remodeling phase, and it is replaced by hollow, robust, and well-organized lamellar bone (Loeffler, Duda, Sass, & Dienelt, 2018; Salhotra, Shah, Levi, & Longaker, 2020).

As a bonus, it can be considered a mineral vault that either releases or stores minerals to stabilize mineral homeostasis. Trauma, illness, malignancies, and anomalies related to aging are the leading causes of bone loss (Harada & Rodan, 2003). Bone restoration is necessary but challenging for patients and clinical surgeons, even if the body can heal and regenerate itself (Stolzing et al., 2008). Therefore, strategies based on various therapies, such as autografts, xenografts, and allografts, have been created to treat bone disorders. The histocompatibility of autografts has made them the treatment of choice for bone defects. Nevertheless, some challenges are associated with these methods, such as postoperative discomfort, significant segmental bone loss, donor site breakage, and morphological mismatch. Fortunately, *in vitro* and *in vivo* research have shown the efficacy of multiple therapy strategies based on different materials, such as drug delivery systems (DDS) or tissue engineering scaffolds. Many diverse biomaterials, including biocompatible hydrogels, inorganic bone cement, and 3D-printed scaffolds, will likely be used to create bone tissue engineering therapy regimens that can meet the needs of patients (Pearson, 2019; Seow et al., 2018; Serati-Nouri et al., 2021; Wan et al., 2022). The extracellular matrix (ECM) is an intricate network structure composed of several biomacromolecules within the cell (Khalili et al., 2022; Pourpirali, Mahmoudnezhad, Oroojalian, Zarghami, & Pilehvar, 2021). ECM serves as a foundational component at a specific place, playing a role in regulating cellular activity, directing the development of new tissues, and establishing links with surrounding institutions (Zamani, Aval, Pilehvar-Soltanahmadi, Nejati-Koshki, & Zarghami, 2018). Hydrogels have a 3D network structure like polymer materials and can expand when exposed to water due to their crosslinked hydrophilic polymer nature. Due to their biomimetic features, hydrogels can create an environment similar to the ECM, stimulating cell differentiation and even tissue development (Grande, Halberstadt, Naughton, Schwartz, & Manji, 1997). As a result of their fascinating biological activity and features, hydrogels have developed as high-potential materials for biomedical applications. Synthetic polymers, hybrid polymers, and organic polymers are only some of the many materials used as the foundation of hydrogel for building bone tissue. Natural hydrogels such as chitosan, collagen, sodium alginate, gelatin, and hyaluronic acid (HA) have gained popularity as biomedical materials for bone healing because of their high biocompatibility. Their utility is constrained by their low mechanical strength, variable biodegradation rate, and risk of immunological reaction (Y. Li, Rodrigues, & Tomas, 2012). Most organically manufactured hydrogels have low stiffness and rapid breakdown since their source components come from living things; this makes them unsuitable for animal models and clinical studies. Another area for improvement is when there are variations in quality across batches throughout manufacturing and processing. Due to these factors, the physicochemical properties of the polymer chain structure may change (e.g., charge, polydispersity, and size). Also, consider that possible immunogenic reactions to these natural materials limit development in the therapeutic domain. As opposed, hydrogels made from synthetic materials like polyvinyl alcohol, polyethylene glycol, polyoxyethylene, polylactic acid, and polycaprolactone (PLA) are characterized by a highly malleable microstructure, a prolonged lifespan, and a robust mechanical strength but lack any biological activity. Natural hydrogels are superior to synthetic hydrogels in controlling cell migration, adhesion, growth, and differentiation in various experimental settings (Jeon et al., 2007). The advantages of naturally produced and synthetically made hydrogels have piqued interest in composite hydrogels merging natural and synthetic components for bone tissue regeneration and engineering. Cell behavior and the rate at which growth factors like transforming growth factor (TGF), bone morphogenetic protein (BMP), fibroblast growth factor (FGF), and insulin-like growth factor (IGF) are released have both been shown to be altered by the addition of inorganic ions or inorganic/organic nanoparticles (NPs) (Xavier et al., 2015). This means that the technique of organic-inorganic hybridization to produce new generations of intelligent hydrogels is already well established. Depending on the type or location of bone disease, composite hydrogels have been developed for various implants, much as how medication delivery systems have changed over the past ten years (such as intravenous injection, cutaneous penetration, and oral administration) (J. Li & Mooney, 2016). With their superior mechanical properties and easily adaptable structure at the predesigned site, hydrogel scaffolds implanted into the body offer unprecedented promise as clinical treatments. Particularly well suited are tissues that serve as load-bearing sites and have large-area defects (Zhai et al., 2017). In situ injectable hydrogels can be employed to repair bone tissues (e.g., slight injuries or cartilage defects) because of their remarkable sol-gel property when filling defect sites, allowing for the avoidance of traditional major surgery (C. Zhao et al., 2019). This review will briefly introduce the variety of biocompatible functional hydrogels used in cell

culture and bone tissue regeneration (Table 1). Many gel design concepts, such as crosslinking procedures, controlled release properties, and alternative bionic methodology, were stressed regarding injectable hydrogels to form bone tissue.

Critical factors of hydrogel material to apply in bone tissue regeneration and engineering

To meet the requirements of bioactivity and biocompatibility in applications, bio and synthetic polymers, as the origin of hydrogels, can be produced from natural molecules in organisms, such as proteins, DNA, and glycosaminoglycans. In addition, various synthetic polymers, including hydroxypropyl methyl cellulose (HPMC), polyvinylpyrrolidone (PVP), and polyethylene glycol (PEG), can be used to create hydrogels as substitutes for natural ones in bone tissue engineering (W. Zhao, Jin, Cong, Liu, & Fu, 2013). Although these biocompatible biomaterials are harmless and barely impair cellular processes, they cannot significantly promote cell migration, differentiation, and proliferation. Discovering additional components enhancing mechanical properties and cell viability would be a fantastic procedure for osteoarthritis, bone defects, or fracture diseases (Sadeghzadeh et al., 2022). A route for mechanotransduction that can control cell migration, choose the final cell type, and prompt the cell to release matching chemicals allows cells to sense the mechanics of scaffold toughness (Harris, Shazly, & Jabbarzadeh, 2013). Therefore, a hydrogel scaffold with suitable mechanical properties in line with genuine bone can improve tissue repair and therapeutic impact. Numerous techniques have been documented to manage the mechanical properties of hydrogels, including using 3D-printed polymer scaffolds as support kernels, mixed nanoparticle systems to control physicochemical properties, and multi-crosslinking network tactics in engineering scaffolds (Gong, Katsuyama, Kurokawa, & Osada, 2003). The number of imported materials (inorganic ions, such as Ca^{2+}, Mg^{2+}, Cu^{2+}, Si^{2+}, and others; nanoparticles, such as carbon nanotubes, mesoporous silicas, gold nanoparticles, and nano-hydroxyapatite; and chemical crosslinking agents, such as genipin, glutaraldehyde, and montmorillonite) can be added to control the bearing capacity (Ayoubi-Joshaghani et al., 2020; Q. Chen et al., 2022; Niazi et al., 2021; Zhang, Yan, Simic, Benetti, & Spencer, 2020). Another critical property that prevents cell migration from native bone tissue or in hydrogel scaffolds is the pore size of the designed platforms. Structures with tiny pores (2–50 nm) can offer a significant surface area for achieving high drug loading efficacy and enhancing protein bioactivity by optimizing the conformation and contact surface (B. Huang et al., 2015). Apatite that resembles bone can be formed, inorganic ion exchange rates can increase, and proteins that compose bone can be absorbed with a middle pore size of 10 micrometers (Karageorgiou &

Table 1 Comparison between the applications of different hydrogels for bone tissue engineering

Types	Characteristics	Application	Reference
Implanted hydrogel	*In vitro* molding, manageable mechanical strength, and easily modifiable shape	Repair of load-bearing bones and cartilage regeneration	(Lankveld et al., 2011; Murphy & Atala, 2014; Tanigo, Takaoka, & Tabata, 2010; D. Zhao et al., 2022)
Injectable hydrogel	Filling irregular flaws, in situ molding *in vivo*, and controllable rheological properties	Bone split, a small bone defect, and cartilage regeneration	(Hao et al., 2022; Hou, Paul, & Shakesheff, 2004; Wasupalli & Verma, 2022; T. Wu et al., 2020; C. Zhao et al., 2019)
Nano/micro gels	Large surface area, adaptable targeting molecules, and flexible injection methods	Osteoarthritis, rheumatoid arthritis, and cartilage regeneration	(Eslahi, Abdorahim, & Simchi, 2016; Heller et al., 2013; S. Li et al., 2018; Shao et al., 2022)

Kaplan, 2005). Cell migration and translation are improved by large pores more significant than 100 m. As a result, choosing a good pore size is essential to speed up the regeneration of bone tissue in hydrogel scaffolds with varying hole sizes (Balakrishnan & Banerjee, 2011). DDS is employed in hydrogel scaffolds for an intelligent release of active chemicals, one of the fields that is receiving much attention (molecule drugs or signaling molecules). To achieve efficient bone tissue healing, it is crucial to create an appropriate method of organization and drug release performance based on the needs of the damage. It usually results in quick drug release activities, which is unfavorable for bone defects needing long-term repair, such as skull damage and cartilage defects, when a drug is directly introduced by modest mixing in the hydrogel. According to reports (Dolati et al., 2016), adding nanoparticles to hydrogel scaffolds that have been functionalized can provide sustained medication delivery to the area of the defect (Tao et al., 2019; Xue et al., 2022a). Target drug delivery, on-demand drug release, and various technologies and theories (such as photothermal technology or magnetic response) are suitable protocols for treating bone disorders, bone injuries, and rheumatoid arthritis (Gaharwar, Peppas, & Khademhosseini, 2014).

Injectable hydrogel scaffolds for bone tissue regeneration and engineering

The benefit of minimally invasive surgery is offered by injectable biomaterials, which have a widespread application in bone regeneration. Concerning filling irregular defects, improving patient compliance in cases of bone injury, and encouraging in situ tissue regrowth and regeneration, injectable hydrogels have several advantages over conventionally performed scaffolds (Hou et al., 2004). Injectable hydrogels can be created using a variety of biomaterials (Fig. 1 and Table 2). Based on the corresponding outcome of the biological evaluation *in vivo*, they all have good biocompatibility and biodegradability. Overall, hydrogels have excellent biocompatibility. To demonstrate this in one study, Samiullah et al. used the free radical polymerization approach and glutaraldehyde to crosslink novel pH-sensitive, biocompatible gelatin/carboxymethyl cellulose-based hydrogels. We examined how pH, polymer ratio, and different crosslinking concentrations affected porosity, sol-gel analysis, *in vitro* release pattern, dynamic swelling, equilibrium swelling, and sol-gel analysis. According to the results, pH 1.2 caused the most edema and medication release. As the polymer load

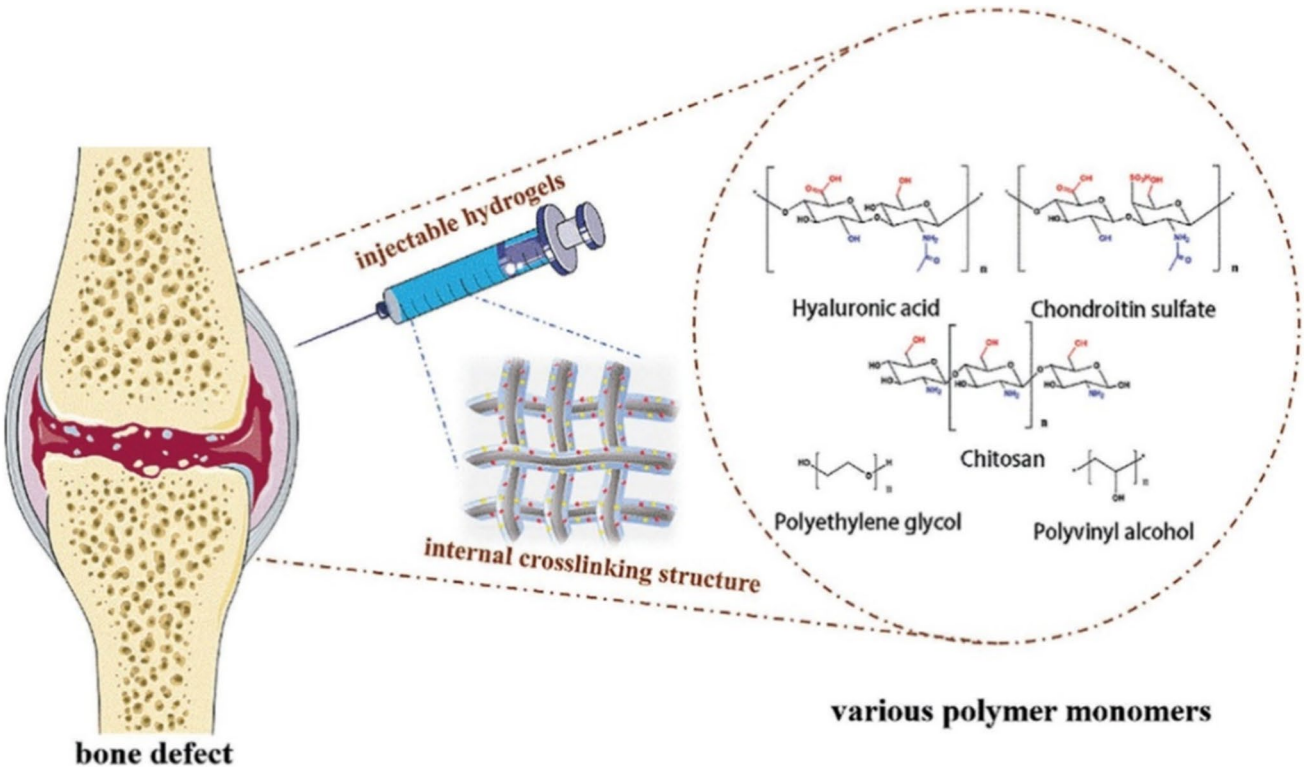

Fig. 1 Polyvinyl alcohol, chitosan, hyaluronic acid, polyethylene glycol, and chondroitin sulfate are biomaterials that can be employed as excellent components of injectable hydrogel for bone tissue engineering. Reproduced with permission (Xue, Hu, Deng, & Su, 2021a). Copyright 2021, Wiley-VCH

Table 2 Injectable hydrogel scaffolds for bone tissue engineering are made of biocompatible materials

Material	Advantages/disadvantage	Method	Application	Performance	Ref
Collagen	Rapid deterioration rate, strong bone conduction activity, and inherent biocompatibility	Triblock PEG-PCLPEG copolymer and nano-hydroxyapatite are chemically linked.	Calvarial defect	Improved biocompatibility, temperature sensitivity, and bone regeneration potential	(Fu et al., 2012)
		Chondroitin sulfate crosslinks itself physically and chemically, self-crosslinks, and forms amide covalent connections.	Chondrocytes	These hydrogels helped chondrocytes remain alive and secrete ECM.	(Gao, Kong, et al., 2018a)
		Chemical crosslinking, genipin, and carbon dot nanoparticle crosslinking	Cartilage regeneration	BMSCs' chondrogenic differentiation and cartilage regeneration should be sped up for in vivo cartilage defect repair	(Xue et al., 2022b)
		Polyethylene glycol is crosslinked twice using chemicals	ATCD-5 cells	Enhanced mechanical and biological properties	(Wei et al., 2019)
Hyaluronic acid	Natural biocompatibility, controllable reactive groups, poor mechanical behavior, and a high rate of deterioration	Chemical crosslinking; dopamine and pluronic F127 catechol-thiol reactions	Cartilage regeneration	Excellent mechanical characteristics; heat response	(Santoveña et al., 2017)
		Chemical crosslinking, tricalcium phosphate, pluronic F127, and the electrovalent and covalent bonding of corn silk extract nanosilver	MSC cells	Desirable antimicrobial activity; increased bone MSC cell differentiation	(Makvandi, Ali, Della Sala, Abdel-Fattah, & Borzacchiello, 2020)
		Creating amide covalent connections between poly(EO-co-Gly)-CHO by chemical crosslinking	Chondrocytes	Increasing the chondrocytes' ability to survive	(Cao et al., 2015)
Chitosan	Tunable reactive groups; good biocompatibility; minimal contact with the cell matrix	Crosslinking using chemical means using Ca^{2+} and Cu^{2+} ions from Cu-bioactive glass nanoparticles	Calvarial bone defect	A successful healing of critical-size calvarial bone lesions in the absence of cells and growth factors	(Yang, Han, Liu, Wu, & Liu, 2022)
PEG	Positive stability, amphiphilic properties, and good biocompatibility, as well as low biological activity	Chemical crosslinking and Ag-SH electrovalent bonding	Intervertebral disk regeneration	Provides potential for intervertebral disk repair while lowering MMP expression	(W. Chen et al., 2020)
PVP	Excellent biodegradability, amphiphilic/soft properties, and minimal biological activity	Crosslinking chemically; crosslinker 4 calcium ion; the 4-carboxy phenyl boronic acid isomer	Cartilage regeneration	Accelerate the regeneration of subchondral bone and cartilage	(Y. Zhao et al., 2018)

grew, the porosity and gel fraction also did. The regulated nature of hydrogels was discovered through *in vivo* absorption and pharmacokinetics testing in rabbit models. The biocompatibility of blank hydrogels against Vero cell lines and their potential cytotoxicity against HeLa cell lines were validated by the MTT experiment. The hydrogel solution is safe up to 4000 mg/kg body weight in rabbits without inducing any hematological or histological alterations, according to the preliminary safety assessment and oral tolerability (Khan & Anwar, 2021).

The physicochemical properties of hydrogels are essential in bone tissue regeneration. The elastic moduli of the brain, muscle, and bone are reportedly 1, 10, and 100 kPa, respectively. It is decided that the exceptional stiffness of hydrogel scaffolds should satisfy the physiological demands in a cell/tissue type-dependent way since hydrogels can provide a specific scaffold structure that resembles the stroma matrix of cell survival for supporting housed cells' activities. Thus, when using hydrogel scaffolds as a substitute for tissue matrix, the stiffness of the hydrogels should be cautiously selected for specific applications (Engler, Sen, Sweeney, & Discher, 2006). Also, by altering hole widths, porous ECM networks have the potential to physically constrain dwelled/traveling cells to variable degrees, as well as have an impact on the multicellular organization and individual cell activity. Hydrogel scaffolds have a porous structure with various pore diameters, another significant physical feature. These pores might influence cellular functions. Since the permeable channels act as the transporting pathways of nutrients, metabolites, and other substances, the porous structure's inconsistency can control a variety of physiological processes and determine whether embedding cells or medications will be successful in treating different types of lesions (Hung et al., 2013; L. Li & Jiang, 2011; Petrie, Koo, & Yamada, 2014). Besides this, it is widely acknowledged that tissues have a feature called viscoelasticity. Moreover, hydrogel biomaterials, which can be made of both ECM-derived and non-ECM-derived materials, exhibit viscoelastic features, such as stress relaxation or creep behavior. These viscoelastic characteristics controlled how housed cells interacted with the surrounding matrix and could cause cell spreading, proliferation, and differentiation changes from cells that were not trapped by the hydrogel scaffold matrix. As a result, controlling the viscoelastic characteristics is crucial for producing a hydrogel matrix that is suitable for the intended use (D. Huang et al., 2019; Maccabi et al., 2018).

Injectable hydrogel based on collagen

A fibrous protein known as collagen has been extensively used in biomedical sectors because of its unique physicochemical and structural characteristics. Collagen can play a significant role in osteoconduction because cells in collagen can move straight into hydrogel scaffolds by integrin. Due to its inherent biocompatibility and ability to stimulate cell differentiation, collagen, a component of cartilage tissue, is an appropriate substance for engineering bone tissue in general and cartilage tissue in particular. In comparison with the hydrogel-based scaffolds with good mechanical properties, the main drawbacks of collagen-based platforms are low mechanical features and quick degradation rate, which limit mesenchymal stem cell differentiation and growth and result in an unmaintainable bone tissue repair effect (Q. Huang et al., 2017). To address the problems mentioned earlier, considerable efforts have been made to improve the performance of collagen-based hydrogels and achieve a healing effect on the bone model. For example, Fu et al., in 2012, effectively created a novel hydrogel based on collagen by fusing collagen, n-HA, and triblock PEG-PCL-PEG polymer (PECE) (Fu et al., 2012). The research inserted the PECE into the collagen chain by covalent crosslinking. Finally, it gave this hydrogel thermal-responsive properties because the unadulterated collagen hydrogel group lacks thermal-sensitive properties. The tempera hydrogel's temperature-sensitive characteristic was investigated through the rheological analysis and tube-test method. The hydrogel had a freedom-of-flow feature at room temperature but started to gel at 37 °C. Additionally, the hydrogel scaffolds were injected for 20 weeks into the cranial lesions of New Zealand White rabbit bone defect models to assess the ability of the bone to regenerate. Histological sections and microcomputed tomography images showed that the group that received hydrogel scaffold injections had a better ability to regenerate bone than the control group. Therefore, PECE/collagen/n-HA hydrogel performed better *in vivo* studies than in the control group regarding bone regeneration capacity and biocompatibility. Another illustration is the injectable, self-crosslinked hydrogel that Gao et al. created using collagen type I. The gel-forming process happens naturally without needing a crosslinking agent and can create a "bionic" environment for cell differentiation and growth. The usual method of preparation is as follows: modified chondroitin sulfate (CS) was first produced by gradually adding N-hydroxysulfosuccinimide sodium salt (sNHS) into the CS aqueous medium; next, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride was added to the over medium under the same reaction conditions. The final step in preparing CS-sNHS was a freeze-drying procedure. The CS-sNHS and Col I were combined in phosphate-buffered saline to create a CS-sNHS/Col I hydrogel solution (PBS). The hydrogel scaffold was produced *in vitro* by injecting the hydrogel precursor solution into a cylindrical mode at 37 °C. It was discovered that altering the amount of substitution of CS-sNHS allowed for simple control of the mechanical feature of hydrogels. According to the findings of the biological evaluation, this hydrogel demonstrated favorable ECM secretory and cell reproductive capacities. Additionally, no overt signs of tissue inflammation were seen in the SD rats' tissue slices after

four weeks of administering the CS-sNHS/Col I precursor solution to Sprague-Dawley (SD) rats. This result proved that injectable hydrogels have outstanding *in vivo* biocompatibility (Gao, Li, et al., 2018b). Smart drug release behavior, controlled porous size, and higher mechanical properties are just a few of the properties of injectable hydrogel scaffolds that can be improved by adding nanomaterials like nanoparticles, nanofibers, nanotubes, and even carbon dots (CDs) (Cui et al., 2018). Additionally, to our knowledge, an excessive amount of reactive oxygen species (ROS) harms the organism, whereas a controlled volume of ROS benefits cell differentiation and growth (Hino et al., 2017). As a result, with carbon dots, reasonable efforts have been made to promote cartilage cell proliferation and provide the best therapeutic impact. To create a flexible injectable collagen hydrogel-genipin-CD-NPs, Lu et al. developed an injectable collagen-based hydrogel for cartilage regeneration. They used conjugated compatible CD-NPs as the active factor to load onto collagen (CGN) (T. Wu et al., 2020). However, it is challenging to acquire good mechanical properties, which must match the support condition of the cartilage by simply adding a crosslinker, genipin. Due to two crosslinking processes produced by genipin and CD-NPs, this GCN hydrogel displayed a challenging stiffness (Xue et al., 2022b). Figure 2 shows the manufacturing procedure for this intriguing injectable hydrogel. The advantage of CGN hydrogels is that they may be treated with an 808 nm

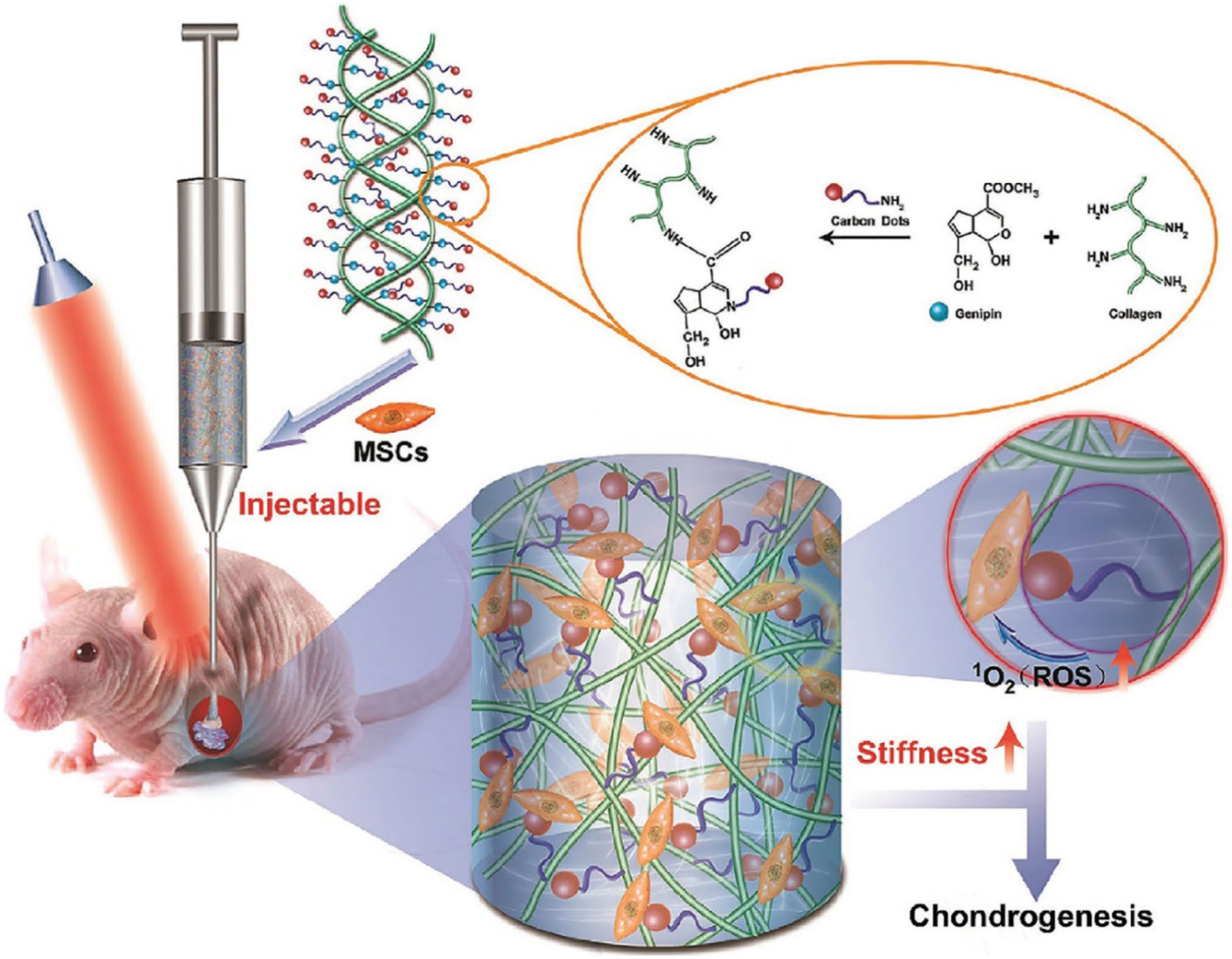

Fig. 2 Collagen-genipin-carbon dot (CGN) nanocomposite hydrogels are made using a general design, showing how they are used. First, a biocompatible crosslinker called genipin was used to bind collagen to carbon dot nanoparticles (CD NPs). Second, the conjugation of CD NPs with the modified collagen resulted in an injectable hydrogel with increased stiffness. Third, the hydrogel containing BMSCs was injected into the cartilage-defective joint. Following hydrogel injection, photodynamic therapy (PDT) was started to produce reactive oxygen species for three minutes every other day at an intensity of 8.3 mW cm^2 for orthotopic cartilage regeneration and 5.6 mW cm^2 for ectopic cartilage regeneration (ROS). The chondrogenic differentiation of BMSCs and, consequently, cartilage regeneration *in vivo* are influenced by the increase in stiffness and the production of ROS. Reproduced with permission (Xue et al., 2022b). Copyright 2019, Elsevier

laser to produce ROS and serve as a suitable crosslinker with less cytotoxicity than gold or silver NPs. Mechanical results showed that CGN hydrogel was more rigid than collagen hydrogel; notably, CGN hydrogel displayed a compression modulus 21 times greater. Its disintegration rate was consequently lower than that of the hydrogel made entirely of collagen. Female SD rats with cartilage defects were treated with 50 microliters of BMSCs-CGN hydrogels for four and eight weeks in an *in vivo* trial using naked female mice and female SD rats. Every other day for three minutes, an 808 nm laser beam is irradiated at the injection site during the treatment to produce ROS consistently. This combination therapy of CGN hydrogel and PDT boosted the BMSC proliferation by about 50.3%. Also, they displayed the most decisive cartilage regeneration impact compared to other groups, as seen from the histopathological and immunohistochemical characterization (CN, C, CGN, and CG groups); these results were statistically different. According to the results of the applicable signaling pathway for cartilage regeneration, TGF-/SMAD signaling and integrin may be involved in mechanotransduction to improve the mechanical features of hydrogel and stimulate the chondrogenesis of BMSCs. These two molecules play critical roles in the development and maintenance of cartilage. Activating signaling pathways like mTOR, which also assisted in chondrogenic development, is associated with ROS regulation. In conclusion, this approach of combining PDT therapy and CGN injectable hydrogel offers a novel way to heal cartilage abnormalities. Hydrogels of collagen repair damaged bone tissue and have vigorous bone conduction activity. However, because of its rapid disintegration rate, it cannot provide long-lasting support for faulty sites. Furthermore, the therapeutic use of collagen in bone regeneration will be hampered by its poor mechanical properties, high cost, and other drawbacks.

Injectable hydrogel based on hyaluronic acid

One crucial glycosaminoglycan (GAGs) in bone tissue is hyaluronic acid (HA) or 1,4-d-glucuronic acid-1,3-N-acetyl-d-glucosamine, which can be the main component of the synovial complex. HA is available and costs less than collagen to design an injectable hydrogel. HA has less immunogenic potential than collagen because it is an endogen. It has been confirmed that HA plays a crucial role in cellular signaling, limiting prostaglandin synthesis, inhibiting proteoglycan release and breakdown, and anti-inflammatory actions (Kim, Mauck, & Burdick, 2011). In addition, hyaluronic acid is a linear macro-polysaccharide with a molecular weight range of 100 to 8000 kDa with accessible N-acetyl groups, COOH, and OH in the structure. Each group's primary goals are to enhance their physiochemical properties and structural organization. Hyaluronidase, an enzyme, and free radicals can also break down HA in living things. In short, adjustable reactive groups and HA have been widely used in bone tissue engineering to treat osteoarthritis, rheumatoid arthritis, and cartilage defects, depending on their biofunctionality (Jeznach, Kołbuk, & Sajkiewicz, 2018). HA's good biodegradability may be a double-edged sword in the application of bone tissue regeneration because of its quick deterioration and weak mechanical action. This can ultimately produce an unsatisfactory bone healing outcome. To make a biocompatible hydrogel with specific mechanical properties, Wang et al. HA-based injectable hydrogel rely on a dual-crosslinking reaction (Xue, Hu, Wang, et al., 2021b; Yu, Cao, Zeng, Zhang, & Chen, 2013). First, by including LAP in the hydrogel system, a covalent link between two furan groups was created when a 365 nm light shone on the surface of the furan-modified HA. Then, in 24 hours at 37 °C, maleimide-modified PEG and furan-modified HA formed a chemical bonding process based on the Diels-Alder reaction. The dual-crosslinking hydrogel scaffold's compression modulus can rise from 5.9 to 21 kPa compared to the single-crosslinking hydrogel scaffold. A dual-crosslinking injectable hydrogel with improved mechanical properties was developed to build a robust platform for bone healing. Although this procedure produced a hydrogel scaffold with good mechanical strength, the increased crossing time prevented hydrogel from being used further. An improved procedure based on the thiol-ene click-on chemical reaction was carried out to shorten the time required for the hydrogel to crosslink (Yazdi et al., 2022). The procedure mentioned earlier created norbornene (HA-Mal-Furan) and SH group-modified HA via covalent bonding. Under UV illumination (365nm; 13 mW cm^2), they could quickly photo-crosslink a hydrogel within two seconds. Its compression modulus increased from 18 to 72 kPa when UV irradiation was extended from 10 to 60 seconds. Additionally, after seven days of growth, ATCD-5 cells were encapsulated within the scaffold and showed good biological activity, but native HA lacked thermo-responsiveness. To increase its thermo-responsiveness and improve its cell adhesive capabilities, HA should naturally conjugate with thermo-responsive polymers such as soluplus, PNIPAAm, gelatin, and pluronic (Ohya, Nakayama, & Matsuda, 2001). Thermo-responsive pluronic F127 and HA hydrogel blends have been used as scaffolds for cartilage regeneration. The hydrogel based on pluronic F127 and HA, loaded with triamcinolone and BPM-2, proved successful in repairing cartilage damage and enhancing the ability to tolerate high loads (Santoveña et al., 2017). As a promising minimally invasive procedure, an injectable hydrogel scaffold can be quickly implanted to cover any shape of bone abnormalities and help patients feel less discomfort (Akkari et al., 2016). However, infections during or following scaffold injection continue to be an unavoidable negative and lower the therapeutic efficiency of bone healing (Nair, Kretlow, Mikos, & Kasper, 2011). Thus,

antibacterial elements such as quaternary ammonium salts, copper ions, and silver nanoparticles (Ag NPs) were added to the injectable scaffold to stop the growth of bacteria. An agricultural byproduct known as corn silk extract (CSE) can prepare Ag NPs by acting as a bioreducing and capping agent. Therefore, it was advantageous to eliminate any other dangerous organic solvent or chemical substance using this green technique. Additionally, an *in vivo* study shows that CSE is effective in healing rat bones that have undergone ovariectomized. A hydrogel composite contains tri-calcium phosphate, hyaluronic acid, and CSE-Ag NPs to obtain green thermosensitive, injectable hydrogel (Makvandi et al., 2020). They produced the Ag NPs using a microwave-assisted technique based on CSE in a medium devoid of organic solvents. The pluronic/HA/tri-calcium phosphate/CSE hydrogels were mixed with Ag NPs to create a thermosensitive scaffold with a sol/gel transition at 37 °C for bone tissue engineering. This novel thermosensitive HA-based hydrogel displayed suitable mechanical characteristics when the gel temperature was higher than the body temperature. Ag NPs, which have a positive charge, can be bonded to the surface of bacterial cell outer membranes and cause structural changes to increase membrane permeability and, ultimately, cell death, giving rise to the desirable antibacterial activity of this hydrogel nanocomposite. Some tests were also conducted to assess the injectable hydrogel nanocomposite's capacity for bone regeneration; pertinent findings showed that the injectable scaffolds promote MSC cell differentiation into bone, making them a promising alternative to the conventional scaffold composite for irregular bone defects.

Injectable hydrogel based on chitosan

Chitosan is another glycosaminoglycan (GAG) found in the cartilage tissue that interacts directly or indirectly with adhesion cells, signal transduction, and growth factors. In addition, chitosan is a polymer compatible with cells and can aid bone rebuilding. Chitosan can easily modify its glucosamine residues (for example, by acylation, carboxymethylation, and alkylation) to enhance its biological events and physicochemical characteristics (Mehrabi et al., 2018). The capacity to form a porous structure is another benefit of chitosan. The porous scaffold's porous structure promotes tissue regeneration and cell migration. Over the past ten years, various techniques, such as redox and photo initiator-guided polymerization, have been used to create a hydrogel that can, in situ, form a durable scaffold. Concern should be expressed about the possibility of cytotoxicity from prolonged exposure to initiators and long radiation (Fedorovich et al., 2009). A mild environment must be established to facilitate the crosslinking of hydrogel networks. The mild Schiff's-base reaction can create imine bonds using amino and aldehyde functional groups (Balakrishnan, Joshi, & Banerjee, 2013). Based on Schiff's reaction, chitosan can be selected as the ideal biomaterial to create a hydrogel due to its structure's abundance of amino groups. To form an injectable hydrogel for cartilage repair, Cao et al. developed an aldehyde-modified PEG, used as a crosslinking factor to interact with the amino functional groups of chitosan. First, chitosan and (EO-co-Gly)-CHO precursors were combined. Then, under mild physiological conditions, this mixture solution formed a covalently crosslinked hydrogel (Cao et al., 2015). Different aldehyde group-modified PEG content levels controlled the in situ hydrogel gelation time and mechanical characteristics. This hydrogel's durability and degradation were demonstrated, and the hydrogel scaffold had a lifetime of up to 12 weeks. Studies on the phenotypic retention, viability, and proliferation of chondrocyte cells in hydrogels were also conducted to assess the hydrogel's therapeutic potential. It proved that covalently crosslinked hydrogels are a promising artificial for cartilage repair and that Schiff's reaction provided an excellent platform for hydrogel in bone tissue engineering. It is not enough to address mechanical strength and gel time alone; instead, the function of materials needs to be highlighted to enhance the therapeutic effect. The vascularization of cambia significantly influences the interaction of regenerated bone with host bone tissue (Quinlan et al., 2015). According to reports, copper ions have been applied as angiogenic promoters for more rapid bone tissue regeneration. According to pertinent studies, Cu^{2+} ions promote angiogenesis and direct stem cells to differentiate into osteoblasts (Burghardt et al., 2015). Cu-doped bioactive glass (Cu-BG) and silk fibroin/chitosan /glycerol phosphate hydrogel CH/silk fibroin (CH/SF/GP) were used to create a cell-free engineering hydrogel by Wu et al. in innovative biomaterials; CH-GP thermal sensitive hydrogels have garnered significant interest lately (J. Wu et al., 2019). Furthermore, the issue of abrupt drug release profiles will be resolved when the drug-loading NPs are added to these hydrogel scaffolds. Therefore, for a long-term outcome on damaged bone, such Cu-BG NPs should be joined to the other slow-release biomaterials and gain sustained release profiles. The incorporation of NPs in hydrogel systems reduces the gel time and increases elasticity, according to tests on gel formation and rheological characteristics. The rat calvarial bone defect was chosen to study and assess the effectiveness of the designed hydrogel. *In vivo* results show an excellent repairing effect in calvarial bone defects.

Other material-based injectable hydrogels

Since PEG has good biocompatibility, excellent stability, and amphiphilic properties, it is mainly used in the biomedical field (Jeong, Bae, & Kim, 2000). An essential part of the resistance to mechanical stress is played by the intervertebral disc (IVD), a cartilage tissue. The overexpression of matrix

metalloproteinases accelerates the breakdown of ECM and leads to IVDD, which is directly linked to intervertebral disk degeneration (IVDD) (Henry, Clouet, Le Bideau, Le Visage, & Guicheux, 2018). Furthermore, IVD can link two nearby vertebrae and has a crucial load-bearing structure. An injectable hydrogel with controllable drug delivery, suitable modulus of elasticity, and self-healing feature is required for local treatment of IVDD to the loading of IVD. To treat this disease, Chen et al. used this design strategy to create a multifunctional injectable hydrogel based on the 4-armSH-PEG (Fig. 3) (W. Chen et al., 2020). A modified miRNA (Agomir) can mimic the capability of miRNA to control the levels of gene expression, thereby reducing the expression of matrix metalloproteinases in the nucleus pulposus. However, due to the risk of leakage associated with its liquid formulation, this gene drug was entrapped in the hydrogel to achieve a long-term drug release and a stable regular microenvironment. This study discovered that combining 4-armSH-PEG

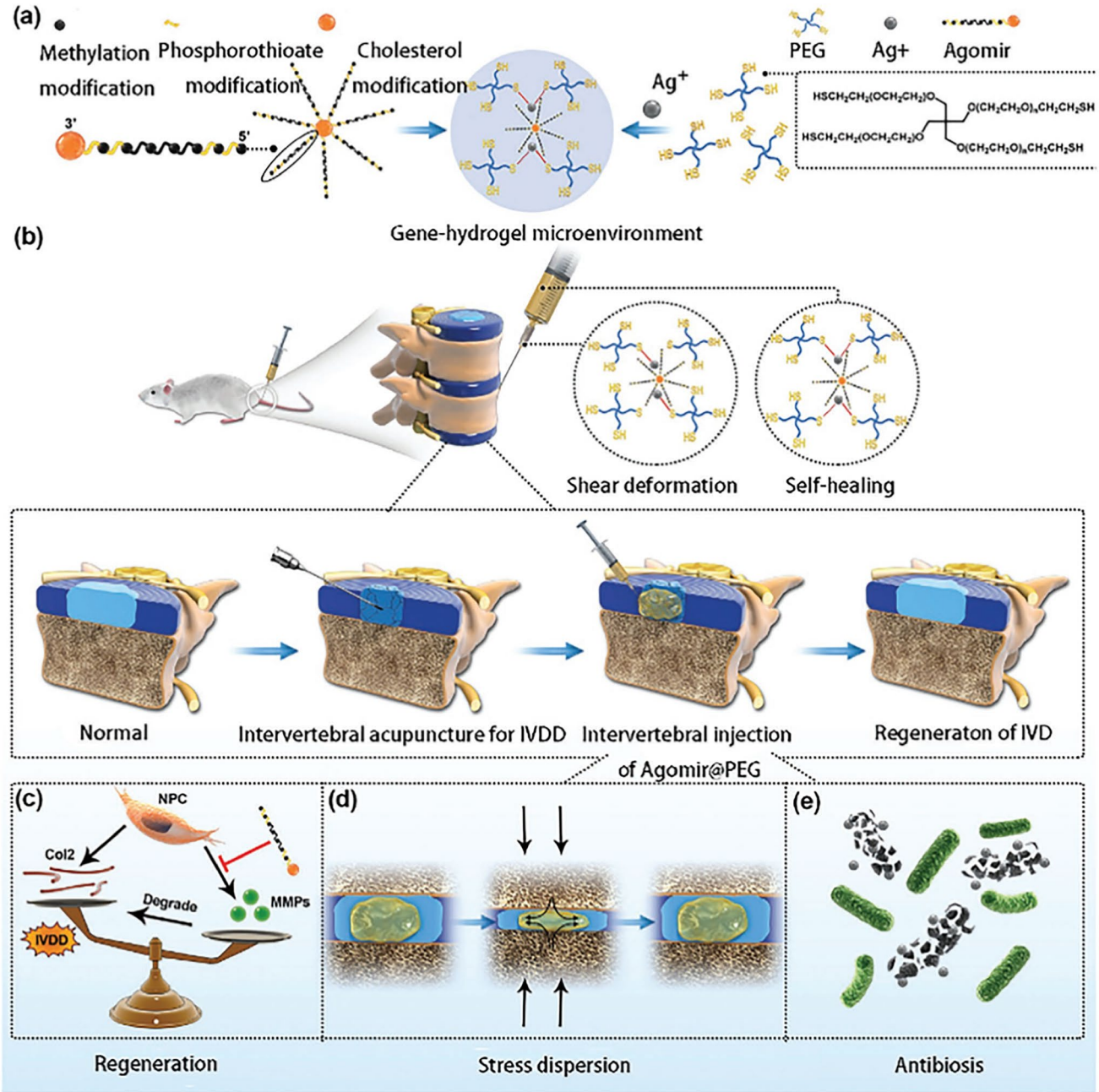

Fig. 3 IVDD regeneration in a gene-hydrogel microenvironment. **a** Building a habitat for genes using hydrogel. **b** Tocreate the gene-hydrogel microenvironment, Agomir@PEG was injected into the intervertebral space. **c**–**e** The gene-hydrogel microenvironment's several roles are compatible with IVDD regeneration. Reproduced with permission (W. Chen et al., 2020). Copyright 2019, Wiley-VCH

with Ag^+ may create an Ag-SH coordination bond. They can naturally form hydrogels with a sticky, glowing foundation without a crosslinking agent.

Additionally, when the shearing force is strong or removed, the Ag-SH will break, and the gel will present a solid state with a viscosity of up to 600 Pa s. This phenomenon demonstrates the hydrogel composite's strong injectability. Even after Agomir 874 was kept in check, the hydrogel maintains its porous, multivoid structure and excellent hygroscopic properties. The cytotoxicity test results showed no significant toxicity, demonstrating the hydrogel's good biocompatibility with cells from different species. In addition, a rat model of IVDD was developed to test this injectable hydrogel *in vivo*. The Agomir 847-loaded Ag-PEG hydrogel demonstrated a better intervertebral disk regenerative capability when compared to the control group, PEG group, and Agomir 874 group in the histological data of the rat intervertebral disk that were obtained from four and eight weeks after the experiment. As a result, this work offers a standard procedure for intervertebral disk repair, resulting in an effective treatment that is also widely applicable to other tissue regeneration. The FDA has approved PVA as another pharmaceutical adjuvant for use as a suspending agent, lubricant, and thickening agent (Muschert, Siepmann, Leclercq, Carlin, & Siepmann, 2009). PVA is currently used in the biomedical industry and has positive tissue regeneration results. However, despite numerous attempts to increase their mechanical strength, PVA-based hydrogels typically have a soft quality. Dual crosslinking hydrogels based on PVA were created by Zhao et al. for cartilage regeneration (Y. Zhao et al., 2018). The 4-carboxyphenylboronic acid (CPBA) was selected as the crosslinking agent. CPBA was added to the PVA precursor, and the sol-gel transition occurred via a borate covalent bond between the CPBA and PVA, forming the gel's crosslinking. The second crosslinking was created by ionic interaction between the calcium ion and the CPBA. Finally, high fracture energy was demonstrated by this dual dynamic crosslinking system against tensile and compression deformation. A rabbit model with a cartilage defect was used to assess the hydrogel's good biocompatibility after injection with PVA. Additionally, histological and immunohistochemical analysis showed that the gel-treated group had a smoother and more complete cartilage layer than the control group. Rutusmita et al. used an in situ gas foaming technique to create a porous biomimetic scaffold. Before it was lyophilized, glutaraldehyde was used to crosslink a mixture of gelatin and PVP. FTIR, XRD, and TGA analyses were used to confirm the mixing of two polymers. The synthetic scaffold was also evaluated for its capacity for osteogenesis and biocompatibility. Investigations were also conducted on physicochemical characteristics such as microarchitecture, porosity, water adsorption capacity, and mechanical strength. Furthermore, the improved migration and proliferation of murine mesenchymal stem cells through the scaffold's interconnected holes over an extended length of time show the scaffold's cytocompatibility. The scaffold's biocompatibility was verified by *in ovo* implantation utilizing a chicken embryo on the chorioallantoic membrane. Alizarin red staining and EDX examination of apatite depositions over the platform further demonstrated enhanced matrix mineralization when triggered with an osteogenic medium. In conclusion, these results show that the gelatin-PVP biomimetic polymer composite scaffold has osteoinductive and biocompatible properties, making it a suitable substitute for bone grafts (Mishra, Varshney, Das, Sircar, & Roy, 2019).

Conclusion and perspective

The ability to produce biocompatible hydrogels for bone tissue engineering has advanced significantly in recent years. These hydrogel scaffolds had excellent therapeutic benefits in bone-related disorders and were implantable in bone tissues via injection, in situ injection, or crude surgical implants. Biomaterials can control drug release profiles in hydrogel or boost mechanical strength by physical and chemical alteration, which can influence the design of hydrogel scaffolds for tissue engineering. Numerous methods have been devised to enhance hydrogel scaffolds for bone tissue engineering. (1) Making hydrogels that are biocompatible, self-repairing, and biodegradable for bone research. (2) Researching the inherent characteristics of hydrogels for cellular adhesion, migration, and expansion in the matrix. (3) To increase hydrogel materials' drug-loading capability and release rate, multinetwork crosslinking hydrogels and nanoparticle composite hydrogels have been produced. (4) Smart hydrogel scaffolds and sensitive-response mechanisms are being developed using cutting-edge synthesis approaches (click chemistry, photo-initiated reaction, molecular assembly strategy, and dynamic physical chemistry). Future studies will need to address practical difficulties. Animal studies *in vitro* or *in vivo* have increased, but clinical investigations have yet to use human models or cells. We know these reasons. (1) Hydrogel-based scaffolds are commonly studied in subcutaneous models that do not represent the multifaceted microenvironment of bone defects and disease. (2) Generally, animal models are healthy and young in evaluating the repairing properties of designed scaffolds. However, clinical bone tissue defect is dominant in older adults. (3) Besides model issues, hydrogel storage is complex, which may hinder clinical use. Swelling hydrogels are easily disrupted during storage and transport, leading to drug leakage. Dehydrating hydrogels after preparation may influence their structure and properties when reswollen. (4) Hydrogels do not self-repair like natural bone tissue. This flaw will harm

hydrogels after implantation or bone activation. (5) Functional hydrogels with good bioactivity and mechanical characteristics have shown well *in vitro* and *in vivo* therapeutic effects. Complicated preparation techniques and high cost limit hydrogel scaffolds' clinical use.

To satisfy clinical objectives, there are still a lot of blind spots in hydrogel design that need to be addressed. Wide hydrogel varieties have been studied in animal models, but only a few hydrogel products based on the human model have been further implemented. These are a few potential issues from a material standpoint. (1) Hydrogel materials are typically implanted subcutaneously to assess the corresponding osteogenic index. However, this method cannot accurately depict the bone defect's microenvironment. (2) The release behavior of the drug will be determined by the uncertainty of the rate of degradation *in vivo* and the interaction between vehicles and bioactive molecules, which will affect the final therapeutic efficacy. (3) Preserving hydrogels, one of the roadblocks in clinical application, is also a challenging problem. Drug leakage may eventually occur because water-contained hydrogels are more susceptible to degradation during storage and transportation. (4) Although many bioactive hydrogel systems for bone tissue regeneration have been developed, there still needs to be a low-cost, all-purpose, and user-friendly technique for using commercial hydrogels. Here, it is anticipated that a more profound comprehension of physical and chemical procedures about the practical design of the hydrogels will be essential to use materials associated with the regeneration of bone entirely. To further integrate the material and the organism, designing sophisticated biomimetic hydrogels can be inspired by research into the fundamental principles underpinning material-biological interactions.

Several essential factors must be considered to improve the therapeutic use of hydrogels. First, hydrogel degradation should match bone defect healing. Second, tissue engineering clinical criteria, safety evaluation, and quality control guidelines for hydrogels should be defined. Patients have different needs, so scientists should pay attention to precision medicine relatively than one-size-fits-all approaches. Bone tissue regeneration is intricate, and each biomaterial has unique advantages, so researchers must select biomaterials for bone repair scaffolds based on application requirements. Combining hydrogel scaffolds with various effective agents (e.g., drugs and cells) may be a promising way to achieve bone engineering. Ideal hydrogel-based scaffolds for bone tissue regeneration require appropriate mechanical properties, easy defect matching, smart drug release, biocompatibility, and biodegradability. Low-cost, cell-free, and easy-to-make platforms should be developed for clinical studies and future human use.

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