Biomaterials Translational

Stimuli-responsive hydrogels for bone tissue engineering

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bone tissue engineering; drug carrier; hydrogels; stimuli-responsive

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ABSTRACT

The treatment of bone defects remains a great clinical challenge. With the development of science and technology, bone tissue engineering technology has emerged, which can mimic the structure and function of natural bone tissues and create solutions for repairing or replacing human bone tissues based on biocompatible materials, cells and bioactive factors. Hydrogels are favoured by researchers due to their high water content, degradability and good biocompatibility. This paper describes the hydrogel sources, roles and applications. According to the different types of stimuli, hydrogels are classified into three categories: physical, chemical and biochemical responses, and the applications of different stimuli-responsive hydrogels in bone tissue engineering are summarised. Stimuli-responsive hydrogels can form a semi-solid with good adhesion based on different physiological environments, which can carry a variety of bone-enhancing bioactive factors, drugs and cells, and have a long retention time in the local area, which is conducive to a long period of controlled release; they can also form a scaffold for constructing tissue repair, which can jointly promote the repair of bone injury sites. However, it also has many defects, such as poor biocompatibility, immunogenicity and mechanical stability. Further studies are still needed in the future to facilitate its clinical translation.

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Introduction

The intricate network of collagen and minerals constructs bone, imparting it with rigidity, strength, and elasticity, playing a pivotal role in supporting the human body.[1] However, developments in science and technology have resulted in an increased incidence of severe trauma, bone tumours, and infections, resulting in troublesome bone defects.[2] While some limited tissue regeneration can occur with adequate blood supply and nutrition, the inherent self-healing capacity of bone has its limits, especially when faced with critical bone defects.[3]

Among the most common transplantation procedures globally, bone grafting stands out as the primary treatment for severe bone defects. Three commonly employed methods include autologous bone grafting, allogeneic bone grafting, and artificial bone grafting. Autologous bone grafting, renowned as the "gold standard", boasts commendable biocompatibility, osteoinduction, osteoconduction, and osteogenic activity.[4, 5] Nevertheless, its application remains constrained by the scarcity of available autologous bone and the adverse effects of donor site damage. While allogeneic bone can replace autologous bone, its osteoinductivity diminishes post-artificial treatment, increasing transplantation failure rates and material extraction complexities. Despite extensive research, no material has yet been comparable to autologous bone. As a response to these challenges, the emergence of bone tissue engineering became a transformative approach. Bone tissue engineering entails the creation of solutions that mimic the structure and function of natural bone tissue, leveraging biocompatible materials, cells, and bioactive factors to repair or replace human bone tissue.

In this realm of bone tissue engineering, hydrogels shine as remarkable gels that retain solubility in water, boasting qualities like high water absorption, elasticity, and biocompatibility, all of which mirror the microenvironment of the extracellular matrix.[6] Hydrogels in bone tissue engineering serve multiple purposes: They act as cell carriers, ensuring the delivery and protection of cells while promoting cell proliferation and differentiation. Moreover, they expertly replicate physical and chemical cues from the biological environment.[7] The true marvel lies in the emergence of stimuli-responsive hydrogels, which dynamically transform in response to the ever-changing *in vivo* and *ex vivo* environments. These hydrogels demonstrate incredible stability and precise targeting, homing in on lesion and injury sites, effectively transporting an array of bone-stimulating bioactive factors, drugs, and cells, all working in tandem to stimulate osteogenesis.[8]

The present review dives deep into the preparation, role, and application of these stimuli-responsive hydrogels, providing a comprehensive account of the latest research progress in bone tissue engineering, focusing on hydrogels with diverse properties. This study showcases the immense potential and widespread opportunities hydrogels offer in bone tissue engineering. Simultaneously, it confronts the key challenges faced by these hydrogels, sparking a thoughtful discussion to pave the way for developing highly versatile hydrogels, thus contributing to the future advancement of bone tissue engineering. We performed a systematic literature search in PubMed and Web of Science databases using the most relevant keywords such as "stimuli-responsive hydrogel", and "bone tissue engineering". It was limited to studies published in English peer-reviewed journals. The main selection criteria measured relevant studies focusing on the application of stimuli-responsive hydrogels in bone tissue engineering and regenerative medicine, with preference given to studies from the last 5 years.

Preparation of Hydrogel

Material sources for hydrogel preparation

Hydrogels, hydrophilic polymer networks, form through covalent or non-covalent cross-linking of polymer chains. Raw materials used include natural sources (chitosan (CS),[9] hyaluronic acid (HA),[10] sodium alginate (Alg),[11] dextran,[12] gelatin[13, 14] and silk fibroin[15]), synthetic compounds (poly(ethylene glycol) (PEG),[16] poly(hash lactone),[17] poly(vinyl alcohol) (PVA),[18] etc.) (**Figure 1**).

Stimuli-responsive hydrogel-derived from natural materials

Natural materials used for hydrogel synthesis are from a wide range of sources with huge natural content, mostly with good biocompatibility, biodegradability, and low immunogenicity. Therefore, they are widely used for the synthesis of stimuli-responsive hydrogels (**Table 1**).[14, 19-27] The following are the stimuli-responsive hydrogels synthesised based on different natural materials.

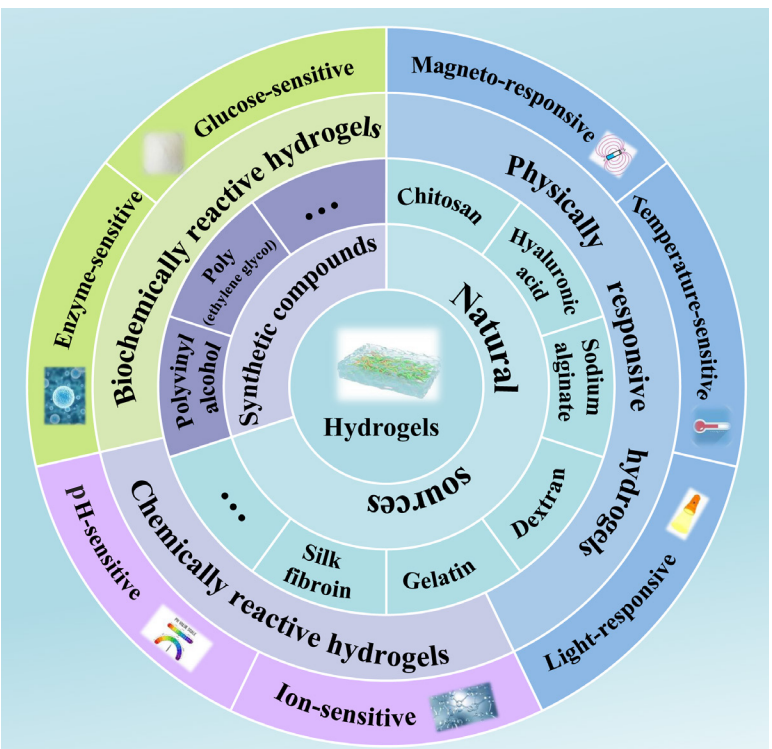

Figure 1. Sources and classification of hydrogel materials. Created with Microsoft PowerPoint 2016.

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Table 1. The use of stimuli-responsive hydrogel-derived from natural materials for bone tissue engineering

Source of hydrogel	Response factor	Application
Chitosan	Temperature	The hydrogel encapsulates adipose-derived stromal cells and maintains their viability, promoting their cartilage formation for the repair of articular cartilage injuries.[19]
	pH	Excellent antibacterial, antioxidant, low cytotoxicity and angiogenic activity. Destroys bacteria, reduces inflammation and promotes angiogenesis to facilitate regeneration and healing of infected wound tissues.[14]
Hyaluronic acid	Light	Provides a supportive environment to support cartilage differentiation and also adapts to the irregular shape of cartilage lesions.[20]
Sodium alginate	Temperature	Slow degradation enables use for long-term release of therapeutic DNA nanoparticles.[21]
		Promoting bone regeneration in a rat cranial defect model, demonstrating the promising application of this hydrogel in unloaded bone regeneration.[22]
Dextran	pH	The hydrogel was able to well encapsulate adriamycin hydrochloride and exhibited a good slow release, while its release showed better behaviour at pH = 4.5 than at neutral pH. The hydrogel can be used as a drug delivery vehicle for the treatment of bone cancer and the promotion of bone repair.[23]
Gelatine	Temperature	Encapsulation of adipose stem cells maintains their viability while continuous release of adipose stem cells due to degradation of gelatine to promote angiogenesis.[24]
		Its hydrophilic properties useful for growth, cell embedding and extracellular matrix secretion can be used as an ideal strategy to promote cartilage tissue formation.[25]
Silk fibronectin	Multiple factors (e.g. pH, temperature and light)	Hydrogel shows excellent stimuli responsive drug release ability and may be used in bone tissue engineering in the future.[26]
	Temperature	It has some osteogenic capacity and can be used for bone repair and in regeneration.[27]

Chitosan-based stimuli-responsive hydrogels

CS is the product of removing some of the acyl groups from the natural polysaccharide CS and is the only alkaline polysaccharide among natural polysaccharides. It contains similar components of the extracellular matrix, which provides a microenvironment for cell growth.[28] The external pH value can influence the protonation/deprotonation of -NH in the CS-based hydrogel, which affects its water solubility and solubility properties. Therefore, CS-based hydrogels are inherently pH-responsive.[29] Tan et al.[14] developed a pH-responsive CS-based hydrogel by cross-linking CS with oxidised dextran via dynamic imine bonding, which exhibits excellent antimicrobial, antioxidant, low cytotoxicity and angiogenic activities. It can destroy bacteria, reduce inflammation, and promote angiogenesis to regenerate and heal infected wound tissues.[14] It can be used to heal infected wounds such as open fractures. In addition, CS has good biodegradability, biocompatibility, non-toxicity, and other biological properties required for bone tissue materials. Tian et al.[30] developed CS-based hydrogels that are bifunctional, thermosensitive and injectable. By modulating the ratio of CS to quaternate CS in the hydrogel, the antimicrobial properties of CS were enhanced and exhibited excellent biocompatibility and mechanical properties. *In vivo* experiments confirmed the good therapeutic effect of the hydrogel in rabbits with infected bone defects. CS-derived stimuli-responsive hydrogels are mostly found in physically and chemically responsive hydrogels, especially in thermo-responsive and pH-responsive types.

Hyaluronic acid-based stimuli-responsive hydrogels

HA is an important component of the intercellular matrix of the human body and is an acidic polysaccharide composed of D-glucuronic acid and N-acetylglucosamine. It is viscoelastic, biocompatible, and biodegradable and also possesses immunomodulatory capabilities. Therefore, HA can be used to load stem cells, providing them with a natural microenvironment. Compared to other materials, HA is more widely available and has a lower immunogenicity.[31] HA-based photo-responsive hydrogels not only provide a supportive environment to support cartilage differentiation, but also adapt to the irregular shape of cartilage lesions. Researchers demonstrated the ability to promote early chondrogenesis by constructing novel cell-free *in situ* photocrosslinked HA hydrogels in an adult minipig model of cartilage defects.[20]

HA has shortcomings, such as rapid decomposition and weak mechanical properties, which can be strengthened by mixing with other materials or by modification. Zheng et al.[32] compounded gelatine/HA photocrosslinked dual network hydrogel with nanohydroxyapatite to form a composite hydrogel. The compression elasticity of this gel was significantly improved, while its fracture strength was close to 1 MPa, and it had good-water holding capacity and biocompatibility, which can be used for the treatment of bone defects.[32] Another study also developed a double crosslinked alendronate-Ca^{2+}/Mg^{2+} doped sulphated HA hydrogel, which contributed to the majority of the hydrogel's mechanical strength, while electroattraction-based cross-linking acts as a release reservoir for Ca^{2+}/Mg^{2+} and alendronate, promoting enhanced osteogenic activity and providing an additional mechanical enhancement to the hydrogel.[33]

Currently, hydrogels based on HA are mostly used for cartilage tissue repair based on their own characteristics. Although it has some drawbacks (poor mechanical properties/prone to decomposition, etc.), the complexation of HA with other materials, combined with the interaction of its own cells and growth factors capable of participating in bone formation, makes it an attractive biomaterial for bone regeneration.[34] Compared to other materials, HA-based hydrogels are able to influence cellular behaviours, including stem cell differentiation, and promote cellular bioactivation.[35] However, their use in the clinic still requires further research.

Sodium alginate-based stimuli-responsive hydrogels

Sodium Alg is derived from the natural polysaccharides of brown algae (e.g., kelp or sargassum), which have high biocompatibility, low immunogenicity, and biodegradability. Alg molecules are composed of β-D-mannuronic acid and α-L-guluronic acid grafted together. Among them, hydrogels with higher guluronic acid content have higher immunogenicity compared to those with high mannuronic acid content.[36] It was found that the stiffness of poly(N-isopropylacrylamide) hydrogels grafted using sodium Alg was directly proportional to the molecular weight of Alg and the ratio of the mannuronate/guluronate monomers. The molecular weight of Alg and the ratio of mannuronate/guluronate monomers also affect the structure, injectability and degradation of the hydrogel. Therefore, the researchers developed thermo-responsive hydrogels than can carry and slowly release therapeutic DNA nanoparticles for sustained delivery of nucleic acids.[21] However, like HA hydrogels, Alg has weak mechanical properties and is often used in combination with other compounds. Shi et al.[22] combined carboxymethyl CS with sodium Alg nanoparticles to construct injectable thermoresponsive hydrogels. Hydrogel. When the hydrogel contained 5 mg/mL of Alg nanoparticles, it was able to promote bone regeneration in a rat cranial defect model, demonstrating the promising application of this hydrogel for unloaded bone regeneration. Other researchers have also constructed hydrogels with pH sensitivity by mixing filipin protein with sodium Alg. It was found that ticlopidine and benadryl exhibited sustained and pH-sensitive release behaviour in this hydrogel, with higher release rates in alkaline environments. Animal experiments revealed that the hydrogel loaded with ticlopidine and benzamil effectively reduced methicillin-resistant *Staphylococcus aureus* infection in rat bone and promoting bone regeneration, providing a considerable approach for the treatment of chronic bone infections.[37]

Dextran-based stimuli-responsive hydrogels

Dextran is a glucose-based unit, with the units connected by glycosidic bonds. It is also a natural polymer and has good biodegradability and biocompatibility. Also, hydrogels prepared from dextran have been shown to provide suitable extracellular matrix conditions and support cell attachment and proliferation.[38] Angelopoulou et al.[23] developed a PH-sensitive dextran hydrogel and used it for the modification of dry hydrogels containing silica. It was found that the hydrogel was able to encapsulate adriamycin hydrochloride well and demonstrated a good slow release, while its release showed better behaviour at pH = 4.5 than at neutral pH. The gel can be used as a drug delivery vehicle for treating bone cancer and promoting bone repair. Based on the inspiration of organic-inorganic analogy in bone tissue and the adhesion chemistry of mussels, the researchers cross-constructed nanocomposite gelatine- and dextran-based hydrogels with self-healing, injectable, adhesive, antioxidant, and osteoinductive properties via Schiff base. Polydopamine-functionalised nanohydroxyapatite was also introduced to improve the interfacial compatibility between the rigid inorganic particles and the flexible hydrogel matrix to enhance the hydrogel network. The hydrogel demonstrated the ability to promote bone regeneration in a rat femoral defect model, and *in vitro* experiments confirmed that it contributed to the osteogenic differentiation of MC3T3-E1 cells.[39]

Gelatine-based stimuli-responsive hydrogels

Gelatine is a naturally occurring polymer made from the hydrolysis of collagen. It is a common structural protein component of the extracellular matrix of the cartilage, bone, tendons, and ligaments. Gelatine also has good biodegradability and compatibility and low immunogenicity, and its ability to spontaneously cross-link hydrogels at low temperatures makes gelatine very versatile in biology.[24] Cheng et al.[40] have developed a thermosensitive CS/gelatine hydrogel that can encapsulate adipose stem cells to maintain their viability and, at the same time, continuously release adipose stem cells due to the degradation of gelatine to promote angiogenesis. Generation. This experiment was confirmed in a mouse wound model. Biodegradable thermosensitive hydrogels ((poly(N-isopropyl acrylamide)-poly(ε-caprolactone) (PCL)-PEG-PCL-poly(N-isopropyl acrylamide)/gelatine and (PCL-PEG-PCL)/gelatine) have also been prepared using thermally-induced phase separation, and their properties have been confirmed through experiments on the characterisation of these hydrogels, their water-absorption capacity, swelling rate, mechanical properties, and co-culture with chondrocytes. Experiments such as co-culturing have confirmed that it has hydrophilic properties beneficial for growth, cell embedding and extracellular matrix secretion and can be used as an ideal strategy to promote cartilage tissue formation.[25] It has also been demonstrated that thermo-sensitive poly(N-isopropyl acrylamide) grafted gelatine has been used by researchers through atom transfer radical polymerisation. Based on the fact that its aqueous solution exhibits a sol-to-gel transition at physiological temperatures, mesenchymal stem cells (MSCs) were encapsulated in it for the regeneration of bone defects.[41]

However, gelatine lacks a stable triple helix structure, resulting in poor mechanical properties and uncontrolled biodegradability. However, since gelatine is resistant to chemical modification, numerous studies have been conducted to improve the properties of gelatine by chemical cross-linking, such as gelatine meth acryloyl hydrogels.[42, 43] In order to mimic the structural integrity and biocompatibility of natural bone, researchers prepared a three-dimensional hybrid hydrogel by mixing gelatine meth acryloyl and sodium Alg. This hydrogel has a high-water content and porous structure, which can facilitate the transport of nutrients during bone regeneration.[44]

Stimuli-responsive hydrogels based on silk fibronectin

Silk fibronectin (SF) are natural polymeric fibrous proteins extracted from silkworm silk, which are enriched with various amino acids (mainly alanine, glycine, and serine, among others).[45] In order to remove the allergic reactions caused by silk glue in silkworms, SF is therefore subjected to a desilk-glue treatment during the preparation process. SF-based hydrogels are multiresponsive or may enable spatial and temporal on-demand drug delivery. Gou et al.[26] developed silk protein hydrogels with injectable, thixotropic and multiresponsive properties. The hydrogel exhibits excellent viscoelasticity and self-healing properties while responding to multiple factors (e.g., pH, temperature, and light). The hydrogel showed excellent stimuli-responsive drug release under different factors and may be used in bone tissue engineering in the future. SF is one of the few natural materials with excellent mechanical strength and physicochemical properties compared to other natural polymers. Therefore, SF is often blended with other materials to improve the mechanical properties of hydrogels. Mirahmadi et al.[46] added SF to thermosensitive CS hydrogels, and the mechanical properties of this hybrid hydrogel were found to be significantly improved by uniaxial compression, indentation, and dynamic mechanical analyses. Yu et al.[27] prepared silica nanoparticles by mixing silica nanoparticles with filipinin and CS, and prepared a covalently crosslinked composite hydrogel with genipin as the cross-linking agent. The hydrogel was thermo-responsive and also proved to have some osteogenic capacity by alkaline phosphatase activity assay, as well as the results of matrix mineralisation in Europe in the cell-gel constructs, which can be used for bone repair and regeneration.

Stimuli-responsive hydrogel-derived from synthetic materials

Despite their many advantages, hydrogels formed from natural materials have certain shortcomings in their use in bone tissue engineering, such as poor mechanical properties and rapid degradation. The emergence of synthetic materials has effectively improved the disadvantages of natural materials (**Table 2**).[47-50]

Poly(ethylene glycol)-based stimuli-responsive hydrogels

PEG is a polymer with good water solubility. PEG has been widely used in bone tissue engineering due to its non-toxicity, non-immunogenicity, good biocompatibility, and resistance to protein adsorption.[51, 52] PEG hydrophilicity has been shown to be controllable, which increases with molecular weight and decreases with temperature.[53] Therefore, some researchers have utilised this property to construct biodegradable thermosensitive PLA-PEG hydrogels. It was mixed with bone cement, and *in vitro* experiments confirmed that the complex had excellent *in vitro* osteogenic properties and had a positive effect on bone repair. It also significantly improved the injectability, washout resistance, and *in vitro* degradability of the bone cement.[47] Some PEG derivatives also have a certain PH sensitivity and can be used to construct hydrogels with pH intelligent response. They can adjust the drug release pattern according to the local microenvironmental pH to adapt to the bone defects in the acidic environment caused by trauma, thus promoting bone repair and regeneration.[48, 54] Although some progress has been made in the research on the enhancement of bone regeneration with the administration of PEG hydrogels, most of the studies are still in the basic experimental stage, and there is a lack of large-scale clinical studies to prove the efficacy and safety of PEG hydrogels, which still needs to be further explored. The effectiveness and safety of PEG hydrogels need to be further explored.

Poly(vinyl alcohol)-based stimuli-responsive hydrogels

PVA is a water-soluble organic compound. PVA has a high modulus of elasticity and excellent bio tribological properties that are biologically similar to cartilage, in addition to good biocompatibility and high-water content. Therefore, PVA has been widely used to prepare hydrogel materials for repairing cartilage defects[55] Meanwhile, it has a promising application in bone tissue engineering.[56] Researchers have developed smart hydrogels capable of responding to reactive oxygen levels. The PVA hydrogel crosslinked by phenylboronic acid is able to degrade with increasing levels of reactive oxygen species, which in turn releases diclofenac sodium encapsulated in the hydrogel, alleviating oxidative stress, reducing post-traumatic inflammation, and promoting cartilage in-growth.[49] Zhang et al.[57] develop reactive hydrogels for inflammatory microenvironments enclosed with quorum-sensing inhibitors,which can treat post-traumatic osteomyelitis by inhibiting quorum sensing and regulating the inflammatory microenvironment, through a reactive oxygen species-responsive bond between N1-(4-borobenzoyl)-N3-(4-borobenzoyl)-the N1, the N1,N3,N3-tetramethylpropane-1,3-diamine and PVA, and the amino side chain of hyperbranched polylysine.

Table 2. The use of stimuli-responsive hydrogel-derived from synthetic materials for bone tissue engineering

Source of hydrogel	Response factor	Application
Poly(ethylene glycol)	Temperature	It has excellent *in vitro* osteogenic properties and has a positive effect on bone repair. It also significantly improves the injectability, washout resistance and *in vitro* degradability of the bone cement.[47]
	pH	The ability to adapt to bone defects in an acidic environment caused by trauma, thus promoting bone repair and regeneration.[48]
Poly(vinyl alcohol)	Activated oxygen levels	Releases diclofenac sodium encapsulated in a hydrogel to reduce oxidative stress, reduce post-traumatic inflammation, and promote cartilage regeneration.[49]
Poly(γ-glutamic acid)	pH	Due to the pH sensitivity, flexible, highly absorbent and smoother hydrogels are produced to serve as useful bone substitutes for repairing bone defects.[50]

Poly(γ-glutamic acid)-based stimuli-responsive hydrogels

Poly(γ-glutamic acid) (γ-PGA) is a water-soluble polyamino acid produced by microbial fermentation and consists of glutamic acid units forming a peptide bond through the α-amino and γ-carboxylic groups. γ-PGA is biocompatible, non-toxic, and biodegradable and is widely used in bone tissue engineering. Yang et al.[58] developed biocompatible γ-PGA hydrogels with strong mechanical properties based on methacrylate-γ-PGA and cysteamine-functionalised γ-PGA. *In vitro* experiments revealed that γ-PGA hydrogels enriched with bone marrow MSCs effectively promoted cartilage formation. Chan et al.[50] synthesised a biocompatible hydrogel by cross-linking calixarene (γ-glutamic acid), sodium Alg and Pluronic F-127, which possessed pH sensitivity. Meanwhile, adding F-127 effectively improves the mechanical properties and affects the temperature-sensitive swelling of hydrogels.

Other synthetic materials-based stimuli-responsive hydrogels

Other synthetic materials can also be used to form stimuli-responsive hydrogels. For example, poly(ethylene) is a tough and strong polymer that is also completely degradable, absorbed and metabolised in living organisms, and has good drug permeability. Therefore, researchers used polyethylene lactone nanoparticles loaded with bispyrimethamine and mixed with CS and filipin protein to prepare injectable thermosensitive hydrogels for bone tissue engineering applications.[59]

Hydrogel cross-linking methods

The commonly used cross-linking methods for hydrogels are physical cross-linking and chemical cross-linking. The physicochemical properties and network structure of hydrogels are different with different cross-linking methods.

Hydrogel prepared by physical cross-linking

Hydrogels prepared by physical cross-linking[60] are mainly formed by ionic interactions, electrostatic interactions, hydrophobic interactions, crystallisation, and their hydrogen bonding, which allows the formation of cross-links between molecular chains through non-covalent forms, such as temperature-sensitive hydrogels.[61] The key factors or conditions for physical cross-linking are mainly concentration, temperature and pH. The cross-linking is mild and therefore the hydrogels formed are mostly reversible and highly adaptable in terms of environment. Its production process avoids toxic organic solvents and small molecule cross-linking agents, and it has the advantages of good biocompatibility and degradability. In order to form hydrogels, physically crosslinked hydrogel polymer networks need to have strong interchain interactions and form stable aggregates in the molecular network; and be able to promote water molecules to enter and stay in the polymer network. The hydrogel has the advantages of non-toxicity, good mechanical properties, strong cell adhesion and slow degradation, and has potential applications in fractures and bone defects.[62]

Hydrogel prepared by chemical cross-linking

Hydrogels prepared by chemical cross-linking form cross-linked networks through chemical reactions between various groups on the polymer chain. The cross-linking reaction can be formed spontaneously *in vivo* (e.g., click chemical cross-linking) or under specific conditions (e.g., photocross-linking reaction). Click chemistry is rapid, spontaneous, versatile and highly selective chemical conjugation reactions, including Diels-Alder reaction, Michael addition reaction or thiol-Michael addition reaction. Due to the mild, safe and effective reaction conditions, their wide application *in vivo* has increased the potential of hydrogels for controlled drug release and facilitated the development of bone tissue engineering.[63] Compared to physical cross-linking, chemical cross-linking improves the flexibility of the cross-linking process and the control of the flexibility of temporal and spatial precision, as well as providing better mechanical strength and stability, making it more suitable for hard and large bone defects.[64]

Conventional Hydrogels in Bone Tissue Engineering

Stem cell mounting or delivery

Harnessing the regenerative power of stem cells, researchers deliver them to bone defect sites, where they stimulate bone repair while managing inflammation.[65,66] The main types of stem cells are bone marrow-derived MSCs, adipose-derived MSCs, and periosteal-derived stem cells. Bone marrow MSCs exhibit superior osteogenesis potential over the cartilage and adipose cell lines, showcasing direct osteogenic effects at stable bone injury sites.[67] Adipose-derived MSCs, with immunosuppressive properties, display promise for anti-immune protection in acute grafts.[68] Periosteal-origin stem cells demonstrate versatile differentiation potential under specific stimuli.

To facilitate stem cell delivery, hydrogels play a pivotal role by promoting cell adhesion at damaged sites, while creating a conducive physical and biochemical environment for stem cell proliferation and differentiation (**Table 3**).[16, 29, 69, 70] Wang et al.[71] observed significant osteogenic effects when hydrogels carried adipose-derived stem cells in a mouse bone defect model. Ren et al.[72] explored how altering HA's molecular weight in hydrogels proved effective for treating bone marrow MSCs. In their study, varying hydrogel strength influenced stem cell activity preservation, with lower strength hydrogels excelling in maintaining stem cell vitality, while higher strength hydrogels induced potential cartilage direction differentiation.

Carrying bioactive factors or drugs

In the intricate process of bone defect repair, a multitude of bioactive factors, including bone morphogenetic proteins and fibroblast growth factors, take centre stage (**Table 4**).[73-77] These factors promote osteoblast proliferation, differentiation, and the formation and mineralisation of new bone at the injury site. Notably, gelatine hydrogel carrying femoral morphogenetic proteins demonstrated its potential to recruit bone progenitor cells and induce new bone formation after subcutaneous implantation, showcasing promising outcomes.[78] Applying fibroblast growth factor locally proved beneficial in enhancing fracture healing and repair, with clinical observations attesting to its efficacy and absence of adverse reactions when delivered through hydrogel. The concentration of fibroblast growth factors exhibited a positive correlation with bone healing progress.[79]

Table 3. Hydrogel loaded with stem cells for bone tissue engineering

Study	Year	Source of hydrogel	Classification	Stem cells	Application model	Mechanisms
Bi et al.[29]	2023	Living active glass/ gellan hydrogel	Temperature-sensitive hydrogels	Bone marrow mesenchymal stem cell	Porcine cartilage defect model	Promote the proliferation of bone marrow mesenchymal stem cells
Filippi et al.[69]	2019	Poly(ethylene glycol)/magnetic nanoparticles	Magneto-responsive hydrogels	Adipose-derived stromal vascular fraction cells	Mice	Alkaline phosphatase activity, expression of osteogenic markers (Runx2, and collagen I) and deposition of mineralised matrix were enhanced. It has the potential to promote osteogenesis and angiogenesis.
Islam et al.[70]	2023	Gelatine	Magnetic-responsive	Adipose-derived mesenchymal stem cells	No	Magnetic fields can control the differentiation of adipose stem cells into different lineages and promote their differentiation into adipose or osteoblasts
Wang et al.[16]	2024	Hyaluronic acid and poly(ethylene glycol)	Environmental stimulus response	Bone marrow mesenchymal stem cells	Rat femoral condylar defect model	Effectively reduces inflammation, accelerates haemoreduction and promotes tissue mineralisation.

Table 4. Hydrogel loaded with bioactive factors or drugs for bone tissue engineering

Study	Year	Source of hydrogel	Classification	Bioactive factors or drugs	Application model	Mechanisms
Madani et al.[73]	2020	Alginate iron hydrogel	Magneto-responsive hydrogels	Bone morphogenetic protein 2	No	Promote the aggregation of osteoprogenitor cells to the defect site, proliferation, and differentiation into osteoblasts
Yao et al.[74]	2021	Gelatine methacryloyl -oxidised sodium alginate hydrogel	pH-sensitive hydrogels	Gentamycin sulfate and phenamil	Mouse calvarial defect model	Promote the repair of large bone defects by enhancing antibacterial activity
Chauhan et al.[75]	2021	Amylopectin - poly(ethylene glycol) hydrogel	pH-sensitive hydrogels	Dexamethasone	Mouse osteoblasts	Enhance cell viability and proliferation, and induce osteoblast differentiation.
Liu et al.[76]	2022	Photocrosslinked chitosan hydrogel	Glucose-sensitive hydrogels	Tannic acid	No	Inhibit the production of NO, IL-6, and TNF-α in stimulated macrophages and exhibits potent antibacterial and anti-inflammatory activities.
Feng et al.[77]	2024	Sodium alginate/ chitosan	Glucose-sensitive hydrogels	Epigallocatechin-3-gallate	No	Good antioxidant and anti-inflammatory properties; modulatory effect on macrophage phenotype, providing a favourable immune microenvironment.

Note: IL-6: interleukin-6; NO: nitrogen monoxide; TNF-α: tumor necrosis factor-α.

To alleviate symptoms and promote bone repair, patients with bone injuries often receive drugs like nonsteroidal anti-inflammatory and analgesics orally, locally, or intravenously. However, these delivery methods may necessitate high doses and repeated administrations, resulting in off-target effects. In contrast, hydrogel-embedded drugs present a superior alternative, allowing direct application to the bone injury site, optimising drug utilisation. Researchers found hydrogel-embedded parathyroid hormone could effectively stimulate cranial bone defect regeneration in mice, exemplifying the potential of hydrogel-based drug delivery in bone repair.[80]

Constructing biological scaffolds

Bone tissue engineering relies on diverse scaffold materials, including metals, bioceramics, hydrogels, and synthetic polymers. Among these, hydrogel scaffolds offer distinct advantages in vascular construction compared to rigid alternatives like metals and bioceramics. This porous network structure of this scaffold is similar to that of the extracellular matrix, which stimulates cardiomyocytes to produce pro-vascular cytokines such as vascular endothelial growth factor, basic growth factor, and transforming growth factor β, and promotes the production of matrix metalloproteinases (MMPs), which in turn degrade the extracellular matrix

and rebuild the vascular structure, which is essential for the regulation of neovascular growth.[81]

Furthermore, hydrogels' soft nature allows customisation to specific applications, matching defect sites precisely, and reducing inflammatory reactions with surrounding cells and soft tissues.[82] Remarkably, three-dimensional bioprinting technology revolutionizes bone tissue engineering, with hydrogel as a commonly used bioink for creating intricate scaffold structures. Anada et al.[83] pioneered the development of a biomimetic hydrogel scaffold by three-dimensional printing, uniformly distributing human umbilical vein endothelial cells and octacalcium phosphate to mimic *in vivo* bone development. Their findings revealed that the hydrogel effectively promoted mouse MSC proliferation, up-regulated alkaline phosphatase expression, and facilitated capillary structure formation *in vitro*. This pioneering work presents a promising implant candidate for bone tissue engineering applications.

Application of Different Stimuli-Responsive Hydrogels in Bone Tissue Engineering

According to the different stimulus properties, hydrogels are divided into three categories: physically responsive, chemically responsive, and biochemically responsive. Physical-responsive hydrogels include magnetic-responsive hydrogels, temperature-sensitive hydrogels, light-responsive hydrogels, etc. Chemical-responsive hydrogels include pH-sensitive hydrogels, ion-sensitive hydrogels, etc. Biochemical-responsive hydrogels include sensitive hydrogels and glucose-sensitive hydrogels. The application of different stimuli-responsive hydrogels in bone tissue engineering are discussed below:

Physically responsive hydrogels in bone tissue engineering

Magnetic-responsive hydrogels

Magneto-responsive hydrogels are the product of combining magnetic nanoparticles[84] with hydrogels, which are able to respond quickly to changes in external magnetic fields[85] (**Figure 2**). Magneto-responsive hydrogels are prepared using hybrid, *in situ* precipitation, or covalent binding methods. Magneto-responsive hydrogels achieve material tracing and targeted release of drugs through an applied magnetic field while regulating the growth, proliferation, and differentiation of stem cells carried by the hydrogel. Filippi et al.[69] researchers found that PEG-based hydrogels containing magnetic nanoparticles remotely driven by a magnetic field had a significant promotional effect on bone regeneration, as well as on angiogenesis. Zhang et al.[86] found that magneto-responsive hydrogels had a significant effect on bone regeneration and angiogenesis, as well as on angiogenesis. Responsive hydrogel-constructed three-dimensional scaffolds exhibited good biocompatibility and could support the attachment and proliferation of human MSCs. At the same time, it can exhibit multimodal movement after the intervention of an external magnetic field. Madani et al.[73] developed a magnetically responsive hydrogel that regulates the release of bone differentiation factors by controlling the timing of external magnetic stimulation, and at the same time the hydrogel promotes the recruitment of bone progenitor cells. Currently, most studies on magnetically responsive hydrogels have focused on *in vitro*, and studies on their *in vivo* biosafety still need to be strengthened.

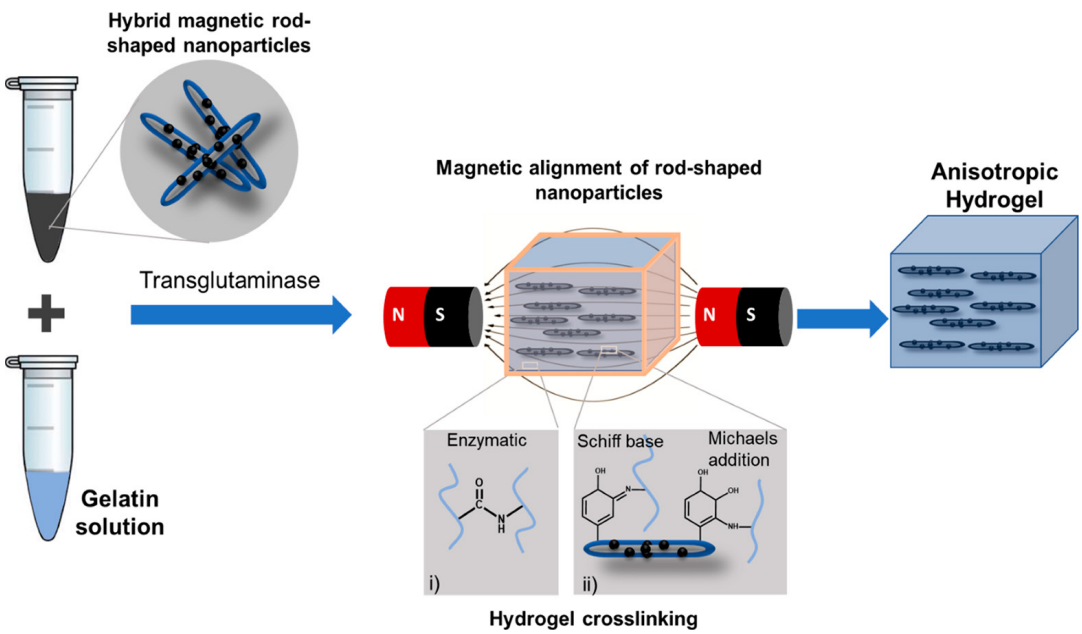

Figure 2. Schematic representation of magneto-responsive hydrogel preparation and cross-linking chemistry. Magnetic manipulation of low concentrations of cellulose nanocrystals decorated with magnetic nanoparticles in enzyme cross-linked gelatine-based hydrogels can be achieved by applying a low intensity magnetic field. Reprinted with permission from Araújo-Custódio et al.[85] Copyright 2019, American Chemical Society. N: north; S: south.

Temperature-sensitive hydrogels

Temperature-sensitive hydrogels can respond to temperature changes and undergo gelation at physiological temperatures to form a three-dimensional structure similar to the bone extracellular matrix for bone tissue engineering applications.[60] While polymers commonly used in the biomedical field are almost not thermosensitive, they are often prepared by covalent cross-linking of thermo-responsive chains with polymers. CS/acrylamide temperature-sensitive hydrogels were found to promote cell proliferation and induce differentiation of human bone marrow MSCs into chondrocytes.[87] Atoufi et al.[88] synthesised a new type of thermosensitive hydrogel of poly(N-isopropylacrylamide)/HA, which contains nanoparticles of poly(lactic acid)-hydroxyacetic acid copolymers coated with CS-acrylic acid and possesses a fast drug release rate, a low rate of separation of the monomers, porous structure, connectivity, high mechanical strength, and bio configuration. The MSCs encapsulated in the hydrogel were found to have normal cell activity and increased glycosaminoglycan synthesis, suggesting that they can be used for cartilage repair. Jing et al.[89] loaded simvastatin into three-dimensional-printed porous Ti6Al4V scaffold for its anti-osteosarcoma and osteogenic effects using thermosensitive poly(lactic-co-ethanolic) acid-PEG-poly(lactic-co-ethanolic) acid hydrogel as a carrier (**Figure 3**). Bi et al.[29] prepared a temperature-sensitive injectable gradient hydrogel using bioactive glass and gellant gum, which was shown to promote the proliferation and induce the proliferation of bone marrow MSCs and the growth of bone marrow cells by cytocompatibility assay and quantitative reverse transcription polymerase chain reaction assay. MSC proliferation, osteogenic and chondrogenic differentiation. Using a porcine cartilage defect model, it was found that irregular osteochondral defects could be seamlessly filled in a minimally invasive manner by injecting temperature-sensitive hydrogels. The temperature-sensitive hydrogel performed significantly better than others in promoting cell proliferation and differentiation and demonstrated superior cartilage repair ability. However, this hydrogel mainly focuses on the cellular level, and the *in vivo* study is limited, requiring many experiments to prove its effect on bone repair.

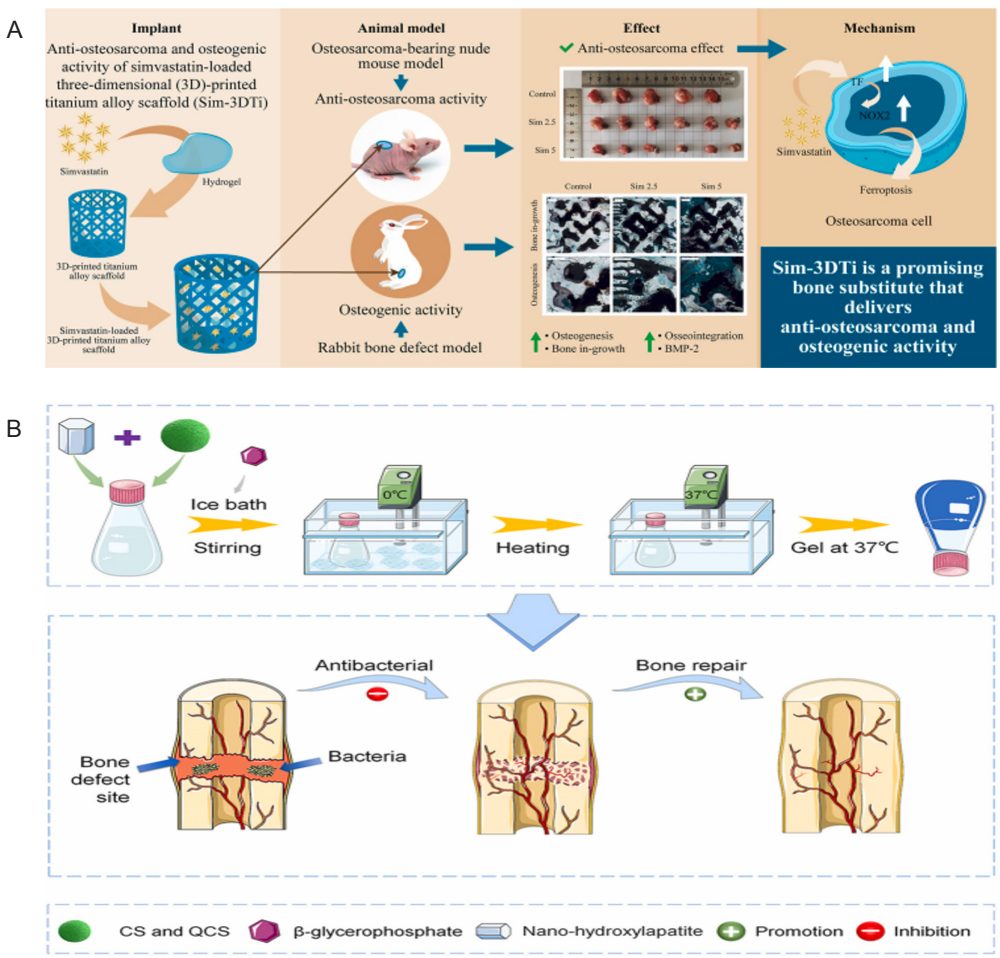

Figure 3. Temperature-sensitive hydrogel for bone tissue engineering cases. (A) Simvastatin was loaded into a 3DTi using a thermosensitive PLGA-PEG-PLGA hydrogel. Reprinted from Jing et al.[89] (B) Bifunctional thermosensitive hydrogel for reducing infection and promoting bone regeneration in infected bone defects. Reprinted from Tian et al.[30] BMP-2: bone morphogenetic protein 2; CS: chitosan; NOX2: reduced nicotinamide adenine dinucleotide phosphate oxidase 2; PEG: poly(ethylene glycol); PLGA: poly(lactic-co-glycolic) acid; QCS: quaternised chitosan; TF: transferrin.

Light-responsive hydrogels

Light can be classified into visible and invisible (ultraviolet and infrared radiation). Visible and infrared radiation light are poorly penetrating and can be absorbed by haemoglobin and water respectively. Ultraviolet light can be absorbed by DNAs and proteins, which can cause radiation-related damage or alter the biological activity of therapeutic drugs.[90] Light-responsive hydrogels[91] use light-responsive groups to convert light into physical or chemical signals, which change the properties of the hydrogel and then modulate the biological behaviours of cells such as cell adhesion, migration, and differentiation, which is one of the most promising hydrogels for bone tissue engineering at present (**Figure 4**).[91, 92] It has been found that near-infrared light-responsive hydrogels can act as antibacterial and anti-inflammatory agents and promote vascularisation and bone regeneration in bone tissue engineering.[93-95] Light-responsive hydrogels have good remote ease of manipulation and high spatio-temporal precision. Tissues change matrix composition and stiffness during development in response to changes in mechanical loading. Although hydrogels can be formulated to change physical properties (e.g., stiffness, etc.), their mechanical properties are fixed at the time of preparation. It has been found that irradiation with near-ultraviolet light (typically ~365 nm) can cause softening of the hydrogel, which subsequently follows changes in cellular behaviour, and can also be used to activate cross-linking of HA methacrylate gels, which leads to stiffening of the substrate, and modulation of the behaviour of the MSCs by altering the substrate mechanics in response to changes in mechanical loading during tissue development.[96] Light-responsive hydrogels use light to trigger the desired mechanical effect without subjecting the cells to chemical stimulation. The range of light to which light-responsive hydrogels respond to light needs to be further developed, thus avoiding the need for harmful ultraviolet light.

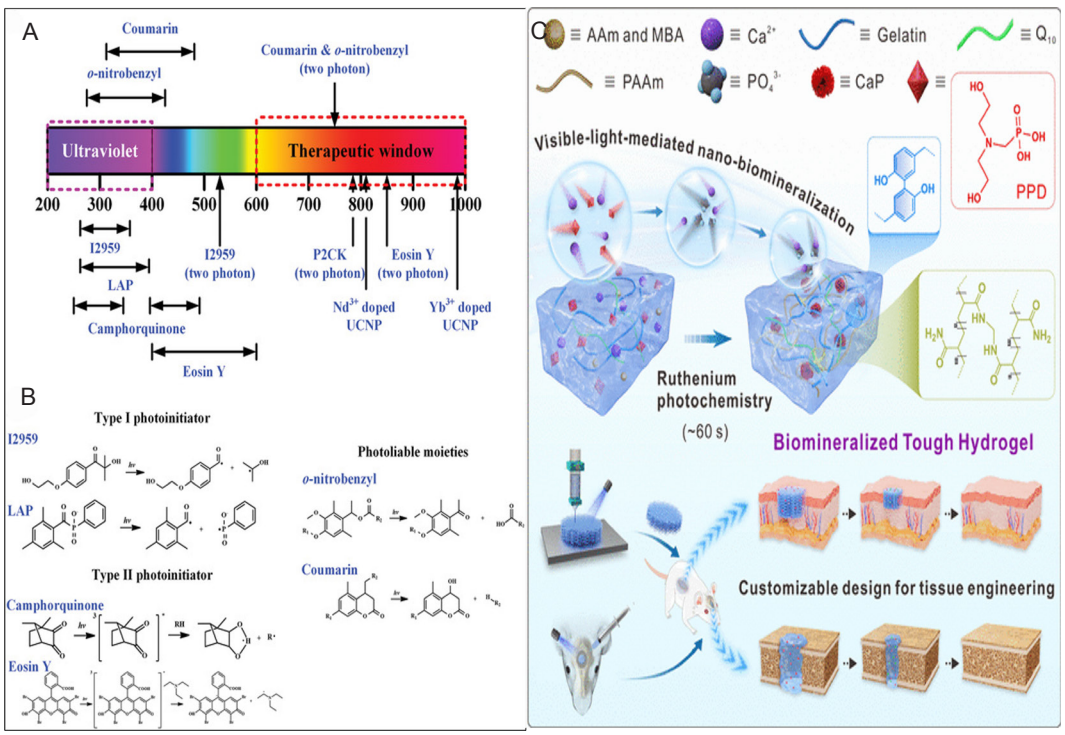

Figure 4. Preparation and application of photosensitive hydrogels. (A) Absorption wavelength distribution of different photosensitizers. (B) Structural formulae of representative photosensitizers. A and B were reprinted from Zhu et al.[91] Copyright WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (C) Schematic for the preparation of customizable BTHs and their application in bionic tissue engineering. Reprinted from Wei et al.[92] Copyright 2022 American Chemical Society. AAm: acrylamide; BTH: biomineralised tough hydrogels; CaP: calcium phosphate; I2959: 2-hydroxy-1-[4-(hydroxy ethoxy)phenyl]-2-methyl-1-propanone; MBA: methylene diacrylamide; Nd: neodymium; P2CK: sodium 3,3′-((((1E,1′E)-(2-oxocyclopentane-1,3-diylidene)-bis(methaneylylidene))-bis(4,1-phenylene))-bis(methylazanediyl))-dipropionate; PAAm: polyacrylamide; PPD: phosphonodiol; UCNP: upconversion nanoparticles; Yb: ytterbium.

Chemically reactive hydrogels in bone tissue engineering

pH-sensitive hydrogels

pH-sensitive hydrogels showcase their adaptability to the varying pH levels[97] in the human environment, transforming into semi-solids with strong adhesion at bone defect sites and precisely delivering drugs to targeted locations. This gel behaviour arises from the abundance of dissociable groups within the hydrogel system, causing mutual repulsion between charges, leading to molecular chain stretching and entanglement. Yao et al.[74] achieved groundbreaking progress by developing pH-sensitive gelatine methacryloyl-oxidised sodium Alg hydrogel. This remarkable hydrogel demonstrated

its ability to carry gentamycin sulfate, effectively enhancing antimicrobial activity. *In vitro* cellular experiments validated its potential in promoting osteogenic differentiation, while *in vivo* studies unveiled its significant impact on new bone formation in a cranial defect mouse model (**Figure 5**). Chauhan et al.[75] introduced injectable polymer hydrogels, employing pH-sensitive and dynamic hydrazone crosslinked oxidised branched-chain starch and octa-armed PEG-hydrazide in a buffer at 37°C. This remarkable innovation paved the way for osteogenic differentiation treatment. Astonishingly, the hydrogel displayed antioxidant, anti-inflammatory, and osteogenic activities, opening avenues for its application in bone repair amidst chronic inflammatory conditions. pH-sensitive hydrogels wield immense potential in bone repair, leveraging targeted drug delivery to precisely combat bone defects, paving the path for more groundbreaking advancements in the future.

Ion sensitive hydrogels

Ion-sensitive hydrogel refers to using polymers to respond to the external ionic strength, and produce reversible changes in structure or conformation, to complete the transition from solution to gel. Deacetylated gellant gum is the most common ion-sensitive polymer. Adult nasal secretions and tears are rich in cations (e.g., Na^+, K^+, and Ca^{2+}), so ion-sensitive hydrogels are more widely used in nasal and ocular preparations. Liang et al.[98] constructed a composite hydrogel with dual PH and salt sensitivity by mixing CS with carrageenan and using epichlorohydrin as a cross-linking agent. It was confirmed that the hydrogel could induce ATDC5 cells to differentiate into cartilage. Kim et al.[99] constructed a methacrylate PEGDA/CS hydrogel using chondroitin sulfate combined with charged ions such as calcium and phosphate. The hydrogel demonstrated a favourable microenvironment for osteogenesis (**Figure 5**).

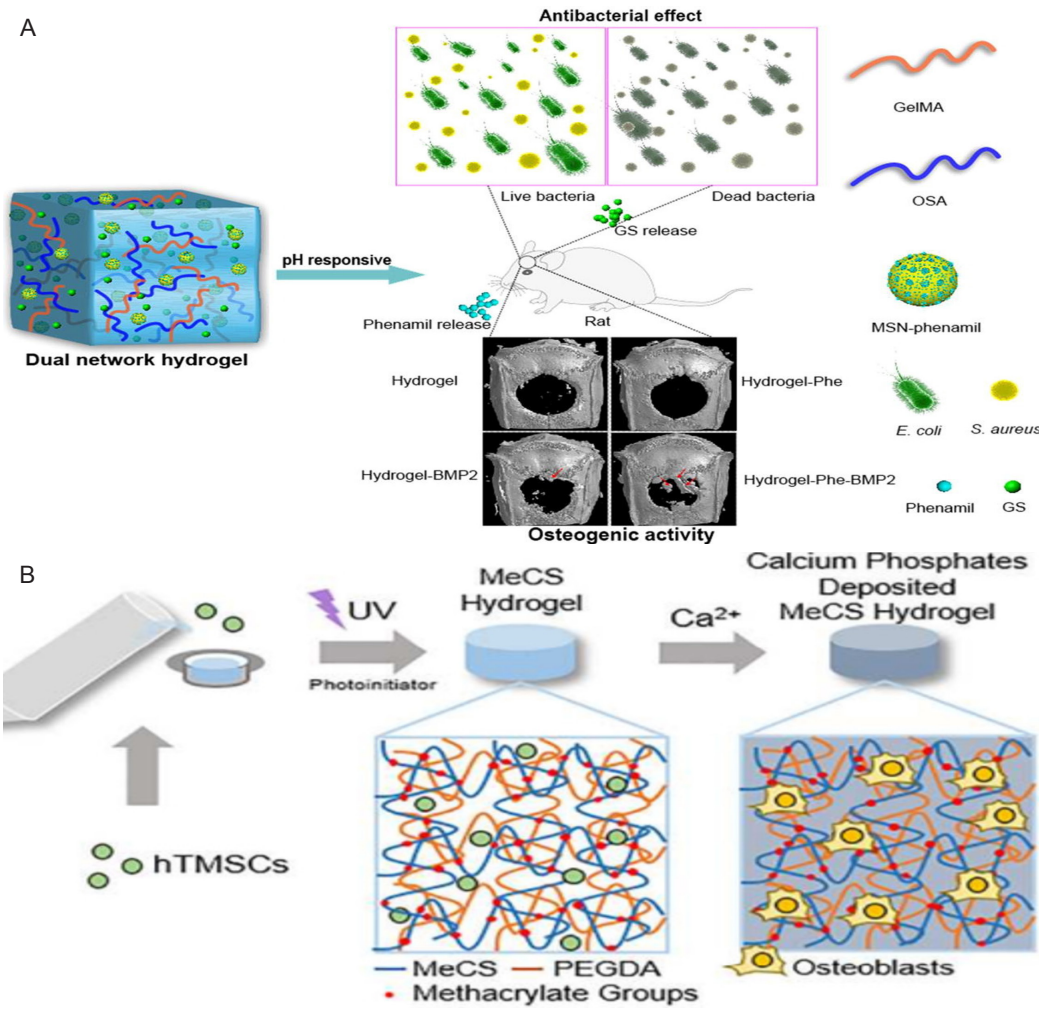

Figure 5. Preparation and application of pH-sensitive hydrogels and ion-sensitive hydrogels. (A) Mechanism of pH-sensitive GelMA OSA hydrogel for dual release of GS and benzylamine (Phe) to enhance antimicrobial activity and promote repair of large bone defects figure. Reprinted from Yao et al.[74] Copyright 2021 Published by Elsevier Inc. (B) Preparation of biomineralised surface hydrogels based on the binding of chondroitin sulphate to charged ions such as calcium and phosphate for bone tissue engineering figure. Reprinted from Kim et al.[99] Copyright 2017 American Chemical Society. *E. coli*: *Escherichia coli*; GelMA: gelatine methacryloyl; GS: gentamicin sulfate; hTMSCs: human mesenchymal stem cells; MeCS: methacrylated chondroitin sulfate; MSN: mesoporous silica nanoparticles; OSA: oxidised sodium alginate; PEGDA: poly(ethylene glycol) diacrylate; *S. aureus*: *Staphylococcus aureus*; UV: ultraviolet.

Biochemically reactive hydrogels in bone tissue engineering

Glucose-sensitive hydrogels

Glucose-sensitive hydrogels stand as a promising avenue for specific and controlled drug delivery, offering self-regulating properties. Responding to changes in the patient's blood glucose concentration, these hydrogels activate the cross-linking network (swelling or shrinking), achieving precise drug release at predetermined rates and times. Xiao et al.'s groundbreaking work[100] introduced a glucose-sensitive CS-polyethylene oxide hydrogel, showcasing exceptional mechanical properties and biocompatibility through meticulous physicochemical and biological evaluations. Crucially, this hydrogel exhibited self-regulated drug release based on environmental glucose stimulation, optimising drug concentration at high glucose levels, making it a potential scaffold for periodontal tissue repair and regeneration (**Figure 6A**). Liu et al.[76] pioneered the synthesis of a glucose-sensitive antimicrobial and anti-inflammatory hydrogel utilising CS with controlled release of tannic acid. This hydrogel demonstrated rapid response to glucose stimulation, influencing the inner hollow structure to effectively control drug release, rendering it highly effective in combating bacterial infections and inflammation. Tseng et al.[101] advanced the frontier with their glucose-sensitive self-repairing hydrogel, offering a vital advantage in achieving vascularisation of bone substitute materials. This novel hydrogel created branching tubular channels within its structure, facilitating the construction of vascularised tissue structures, particularly neurovascular units crucial for bone tissue regeneration. Given the escalating incidence of diabetes mellitus, non-healing bone conditions caused by changes in blood glucose become increasingly prevalent. While glucose-sensitive hydrogels currently find extensive use in oral diseases, they hold immense potential in bone tissue engineering. However, challenges like mechanical compatibility, biocompatibility, and drug-controlled release capabilities necessitate further investigation to pave the way for enhanced clinical applications. The future awaits transformative breakthroughs in this captivating field.

Enzyme-sensitive hydrogels

Enzyme-sensitive hydrogels, employing physical or chemical means to link peptide molecules that react with specific enzymes, usher in a cascade of reactions, culminating in gel formation. Fascinatingly, PEG with the peptide cross-linker C-VPLS↓LYSG-C (a bis-cysteine crosslinker) facilitated the creation of hydrogels sensitive to MMP-2 and MMP-9.[102] These hydrogels displayed a unique trait of being readily degraded *in vitro* while resisting rapid degradation due to foreign body reactions, as confirmed through *in vitro* and *in vivo* experiments. Furthermore, encapsulated MSCs demonstrated their ability to degrade the hydrogels using MMPs, bestowing long-term stability *in vivo* and evoking promise for bone tissue engineering applications. Intriguing breakthroughs continued as Schneider et al.[103] designed MMP-sensitive PEG hydrogels, skilfully encapsulating chondrocytes to dissect the hydrogel-to-new tissue transition process. Unveiling an intimate relationship between new tissue growth and hydrogel degradation, the MMP-sensitive hydrogel rapidly fostered ECM growth within cell clusters, nurturing the growth of newborn cartilage. Zhang et al.[104] constructed an MMP-responsive injectable hydrogel by connecting an MMP cleavable peptide to a tetra-PEG network assembled from PS molecules. The hydrogel was able to respond to changes in MMP by releasing large amounts of PS early in inflammation and sustained retention of drug release until late stages of bone repair. *In vivo* and *in vitro* studies further demonstrated the ability of PEG-peptide-phosphatidylserine to convert macrophages to an anti-inflammatory M2 phenotype and promote osteogenic differentiation, leading to new bone regeneration (**Figure 6B**).

Yet, the presence of a reaction-diffusion mechanism lends complexity to the spatiotemporal degradation behaviour of enzyme-sensitive hydrogels. As the journey progresses, further studies delve into crafting enzyme-sensitive hydrogels with controlled degradation, manipulating initial hydrogel properties or enzyme kinetics to unveil even more remarkable possibilities in bone tissue engineering. The path ahead is riddled with uncharted territories, awaiting bold explorations and transformative advancements.

Conclusion

In the past years, a remarkable surge in stimuli-responsive hydrogel research, coupled with the rapid advancement in polymer science, pharmacy, and biomedical engineering, has given rise to the emergence of multiple-responsive hydrogels tailored for diverse lesion environments. These versatile hydrogels have gained paramount significance in the realm of bone tissue engineering.

This comprehensive review amalgamates the research findings of fellow scholars concerning the raw materials, mechanical properties, and biological attributes of stimuli-responsive hydrogels. Delving deep into the experimental studies, it meticulously analyses and compares the performance, advantages, and drawbacks of these hydrogels, presenting diverse scenarios for their applicability. However, these studies are in the early stages and there are many limitations before they can be applied to the clinic. Firstly, the stability of the triggering conditions limits its application, and it is necessary to develop new and stable stimulation methods. Second, the degradation rate of hydrogels does not match the regeneration rate of bone defects; in addition, the poor biocompatibility, immunogenicity, and mechanical stability of some hydrogels limit their use in the clinic. Given the intricacy of tissues, a one-size-fits-all hydrogel for bone defect repair remains elusive. To realize personalised clinical applications, the need to develop complex, multi-stimuli-responsive hydrogels looms large. Currently, among all stimuli-responsive hydrogels, temperature-sensitive hydrogels are the class of hydrogels with the greatest potential for clinical use due to their ability to form hydrogels at physiological temperatures without the need for special manipulation and functional modification; and their simple composition, low cytotoxicity, and ease of industrial production.

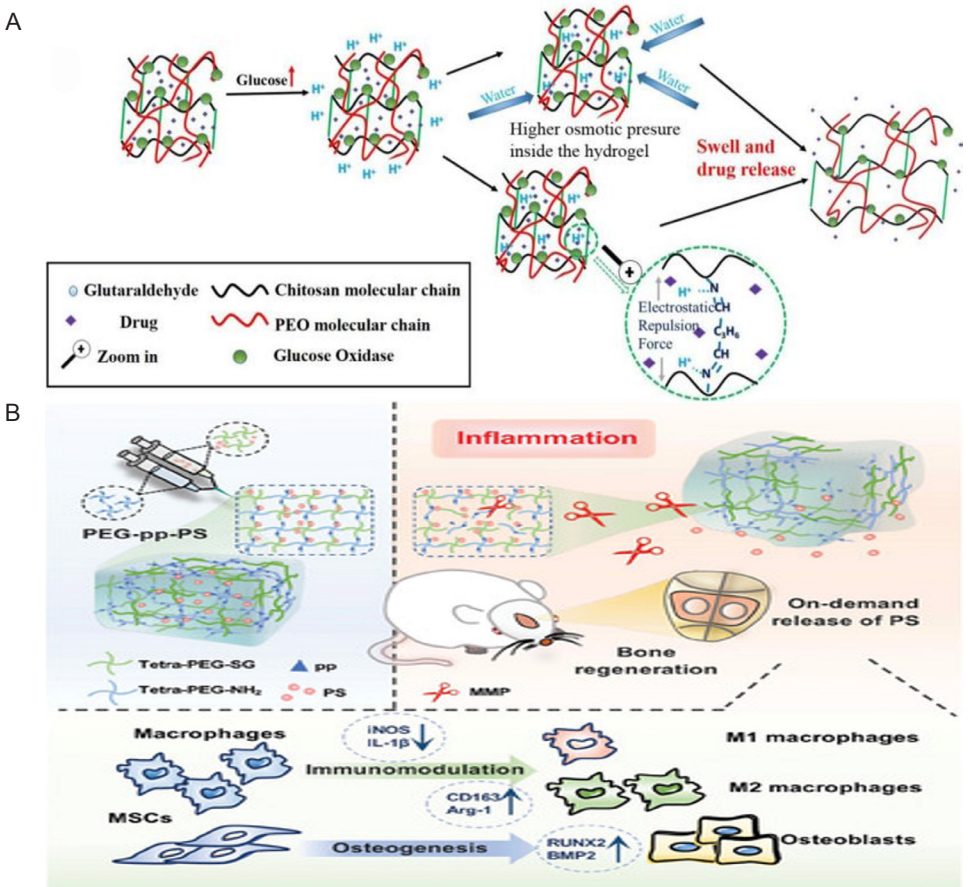

Figure 6. Glucose-sensitive hydrogels and enzyme-sensitive hydrogels. (A) The mechanism of chitosan-PEO hydrogel's glucose sensitivity and controlled drug release process. Reprinted from Xiao et al.[100] Copyright 2015 Elsevier Ltd. (B) Schematic diagram showing the fabrication procedures of a MMP-responsive phosphatidylserine (PS)-encapsulated injectable hydrogel (PEG-pp-PS) for rat calvaria bone defect regeneration. Reprinted from Zhang et al.[104] Arg-1: arginase-1; BMP2: bone morphogenetic protein 2; IL-1β: interleukin-1β; iNOS: inducible nitric oxide synthase; MMP: matrix-metalloproteinase; MSCs: mesenchymal stem cells; PEO: polyethylene oxide; pp: peptide; RUNX2: Runt-related transcription factor 2; SG: succinimidyl glutarate.

Despite promising prospects, several bottlenecks impede progress. Deeper, more systematic research into rheology, mechanical properties, response rate, *in vivo* behaviour, and safety is crucial. Addressing the poor biocompatibility of certain hydrogels and fine-tuning drug release rates for precise lesion or specific release site targeting are among the challenges. Striving to achieve the vision of precise, individualised, and intelligent medical care, the future holds boundless potential for stimuli-responsive hydrogels.

In this era of transformative advancements, stimuli-responsive hydrogels are poised to shape the face of precise, individualised, and intelligent medical treatment, transcending barriers and unlocking innovative therapeutic horizons. The journey ahead promises unparalleled innovation and groundbreaking discoveries, propelling the realm of bone tissue engineering into uncharted territories.

Author contributions

CX, XL, LX and RK conceptualised and designed the review; CX, NW and HC drafted the manuscript; ZX, WX and LC checked and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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Conflicts of interest statement

The authors declare no conflict of interest.

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