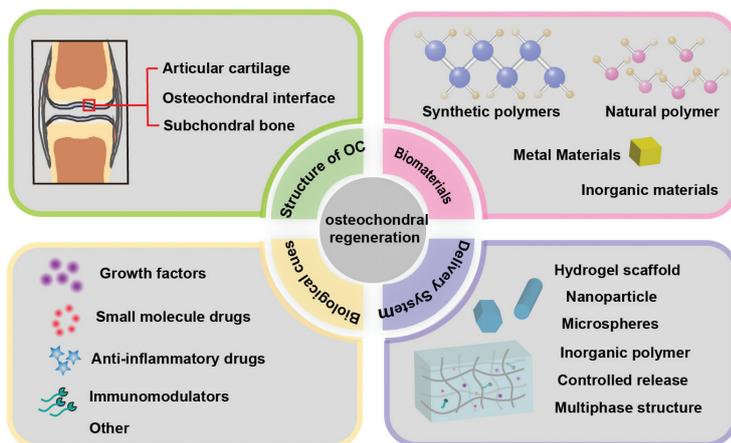


# Advances in Drug Delivery Systems for Osteochondral Tissue Regeneration

## Graphical abstract



## Highlights

- Osteochondral regeneration faces challenges due to cartilage's avascularity and poor self-repair.
- Biomaterial-based delivery systems (hydrogels, nanoparticles, multiphase scaffolds) enable spatiotemporal control of bioactive cues (growth factors, chondroinductive small molecules).
- These systems enhance penetration, prolong retention, and promote simultaneous cartilage/subchondral bone repair.
- This paper systematically reviews the latest advancements and explores the great potential of biomaterials in drug delivery systems for bone and cartilage regeneration.
- It provides compelling evidence for bone and cartilage tissue regeneration in both basic and clinical research.

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## In brief

In this paper, the biomaterial delivery systems developed in recent years have been reviewed to identify the key factors for the success of OC regeneration. Key factors for successful OC regeneration have been considered in this review, as follows: i) biological structure of osteochondral units; ii) types and combinations of biomaterials; iii) drug of choice; and iv) commonly used delivery systems.

# Advances in Drug Delivery Systems for Osteochondral Tissue Regeneration

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## Abstract

Osteochondral damage is primarily caused by degeneration of articular cartilage due to trauma, degenerative injury. Current approaches to treating cartilage damage typically involve medications, physical therapy, interventional therapy, and surgery. Drug delivery to the cartilage has shown promise in cartilage regeneration and slowing the progression of cartilage degeneration. However, the unique physiologic composition of articular cartilage poses challenges for effective drug delivery. Systemic administration of drugs may lead to inadequate drug distribution within the cartilage. Direct intra-articular injection of drugs can result in rapid clearance and limited retention time of the drug in the joint cavity. In summary, the limited duration of drug efficacy within the joint due to a short half-life and restricted diffusion through cartilage poses challenges for long-term drug therapy. Surgical intervention becomes necessary in cases of severe joint degeneration or ineffectiveness of alternative treatments. Various clinical studies have demonstrated the effectiveness of surgical approaches, such as an osteochondral autograft transplantation system (OATS), autologous chondrocyte implantation (ACI), and microfracture. Unfortunately, these interventions are also associated with potential adverse effects. The ongoing advances in biomaterials for osteochondral tissue regeneration and replacement have led to a growing interest in the utilization of biomaterial-based drug delivery systems for tissue regeneration. The integration of bioactive molecules, drugs, and genes with biomaterials presents a novel strategy for advancing osteochondral regeneration. A desirable drug delivery system should be able to penetrate cartilage tissue before the drug is cleared by synovial capillaries and lymphatic vessels, and bind to components of the cartilage extracellular matrix to reduce the physical deformation of cartilage during locomotion, resulting in a cartilage-specific drug pool. This paper was motivated by the great potential of biomaterials for osteochondral tissue regeneration and reviews recent progress in biomaterial-based drug delivery systems for osteochondral regeneration.

## Keywords

bioactive molecules, delivery system, osteochondral regeneration, osteochondral unit.

## Introduction

The connective tissue that covers the bony ends of human joints is termed as articular cartilage. The high-water content of articular cartilage helps lubricate the joint and absorb shocks and the collagen strands within articular cartilage provide strength and elasticity. Articular cartilage has the primary function of lubricating the joint and reducing stress during movement [1, 2].

When the osteochondral (OC) unit is damaged due to trauma, degenerative lesions, severe pain and restricted movement can occur. Young individuals might experience cartilage damage due to sports injuries, whereas older adults often have cartilage damage due to the natural deterioration of joints. Factors that increase the risk of cartilage damage include being

overweight, a previous joint injury, and some genetic predispositions. The most prevalent type of cartilage damage is associated with osteoarthritis, which results in degenerative changes and breakdown of the articular cartilage [3]. Damage to cartilage can cause degeneration of joints, potentially leading to severe disabilities and significantly impacting patient well-being [3–5].

Classification of OC defects ranges from grade I, which is nearly normal, to grade IV, which indicates a severe abnormality. This grading system is based on the depth of the lesion and the components of the OC unit affected. Specifically, the grading system assesses whether the damage is limited to the cartilage or extends to the cartilage and subchondral bone, which comprise the entire OC unit [6]. It is difficult to regenerate cartilage tissue due to the special structure of cartilage and the lack of blood vessels and lymphatic vessels

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[7]. Currently, there are several treatment methods for OC repair, including drug therapy, physical therapy, and surgical intervention [8, 9]. Medications, such as non-steroidal anti-inflammatory drugs (NSAIDs), can reduce inflammation and relieve pain. Physical therapy may also improve the strength and flexibility of the affected joint. However, surgical treatment is the last resort treatment option when the joint is severely diseased or does not respond to alternative treatments. Current surgical treatments for OC defects include chondroplasty, joint replacement, the OC autograft transplantation system (OATS), autologous chondrocyte implantation (ACI), and microfracture [10]. However, chondroplasty can only be palliative. While microfracture treatment can temporarily enhance joint function, microfracture treatment frequently result in the formation of fibrous tissue, weakening of the subchondral bone, and a decline the joint functionality over time [11, 12]. ACI involves performing a cartilage biopsy from patients at non-weight-bearing sites, isolating and expanding the chondrocytes *in vitro*, reimplanting the chondrocytes into the defect, and using periosteal flaps to maintain the implanted chondrocytes *in situ*. However, this procedure requires two surgical procedures, requires a prolonged recovery time, and frequently has complications due to flap hypertrophy [13]. Microfracture surgery remains a first-line intervention for small-to-medium cartilage defects, demonstrating significant short-term functional improvement and pain reduction within 6–12 months. Histologic studies have confirmed increased cartilage volume via fibrocartilage formation. However, Kreuz et al. reported the following critical long-term limitation: repaired tissue exhibits biomechanical inferiority, leading to progressive degeneration under physiologic loads [8]. This limitation manifests clinically as a functional decline in 68% of patients within 18–24 months postoperatively. Microfracture surgery offers cost-effective arthroscopic management of small-to-medium cartilage defects with low complication rates and rapid recovery. However, the long-term efficacy of microfracture surgery is limited. This approach is contraindicated for defects > 4 cm<sup>2</sup> in size, obese patients (BMI > 30 kg/m<sup>2</sup>), or mal-aligned joints. The OATS remains the gold standard for defects ≤ 10 cm<sup>2</sup> in size. The OATS restores hyaline cartilage (type II collagen > 90%) with superior integration strength, though the OATS requires open surgery and carries donor-site morbidity risks. The OATS is also recommended for patients with larger defects or significant joint degeneration [8, 9]. The OATS can improve the prognosis, including a reduction in pain and improvement in function. The OATS has a high long-term success rate and provides good mechanical strength. However, the OATS is an invasive surgical procedure that requires a second donor tissue surgical site. The OATS has a high risk of adverse events related to the donor site and a long recovery time. ACI can effectively repair cartilage defects, improve joint function, and can be used for large cartilage injuries. ACI can provide good mechanical strength and does not require a second surgical site. However, this process requires advanced technical skills, specialized equipment, and professional knowledge. The risk of complications related to the implantation process is high and recovery takes a relatively long time [14, 15]. In conclusion, OC regeneration is a major medical hurdle

because of the diverse physiologic characteristics and inherent structures of cartilage and subchondral bone.

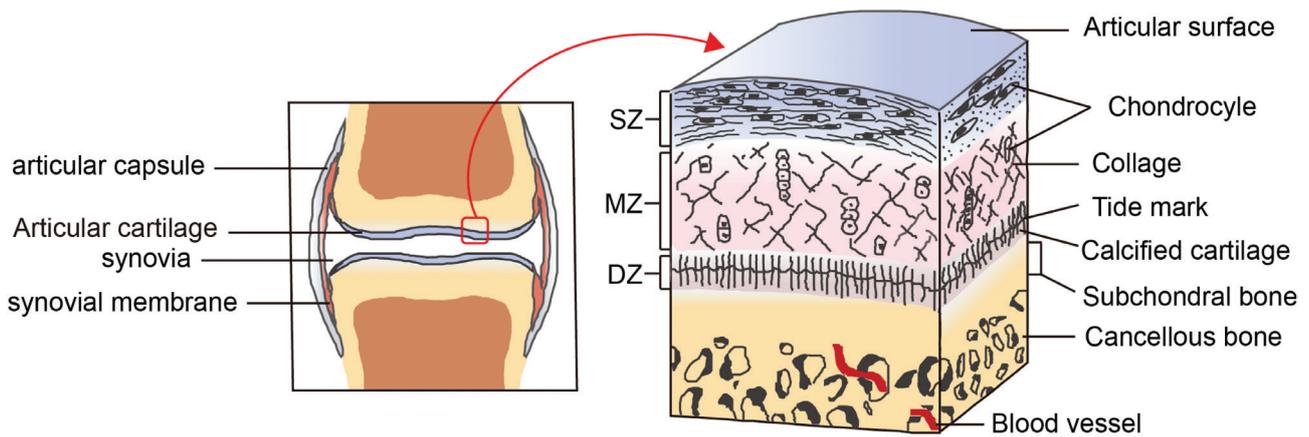
Improvements in OC regeneration have been achieved in recent years by combining biological components and biomaterials with suitable biomechanical characteristics and structures. Combining biological components and biomaterials provides a novel therapeutic strategy for restoring, maintaining, or improving the function of OC tissue. Combining biological elements with tissue engineering principles to combine different substrates, cells, and bioactive molecules as vehicles for drug and/or cellular delivery may open new prospects for OC regeneration. In this paper the biomaterial delivery systems developed in recent years have been reviewed to identify the key factors for the success of OC regeneration. Key factors for successful OC regeneration have been considered in this review, as follows: i) biological structure of osteochondral units; ii) types and combinations of biomaterials; iii) drug of choice; and iv) commonly used delivery systems. Based on this careful analysis, promising directions for the treatment of OC regeneration, which remains one of the most formidable clinical problems in orthopedics and rheumatology, will be discussed.

## Biological structure of OC units

The OC unit is a holistic functional entity consisting of articular cartilage, subchondral bone tissue, and osteochondral interfaces, as shown in **Figure 1**. The structural and functional properties of OC units vary.

### Articular cartilage

Articular cartilage is situated on the joint surface and consists of hyaline cartilage, which is known for its elasticity and smooth surface. Articular cartilage acts as a buffer during joint movement and reduces friction between adjacent bones and transfers the mechanical load to subchondral bone [1]. Cartilage tissue has a low cellular density and primarily consists of chondrocytes, which accounts for approximately 2% of the total volume. Chondrocytes aggregate into “cell nests” or “homologous groups” and infiltrate the abundant extracellular matrix (ECM) [16, 17]. Chondrocytes have an essential role in joint stability. The hyaline cartilage ECM is organized into four distinct structural and biochemical regions (surface, middle, deep, and calcified regions), all of which contribute to viscoelastic properties [1]. The ECM is predominantly comprised of collagen (specifically, type II collagen) and proteoglycans with lesser quantities of adherens glycoproteins and elastin fibres [18]. Proteoglycans have a vital role in chondrocyte metabolism [19]. The invasion and differentiation of exogenous progenitor cells is unfavorable due to an extremely limited number of endogenous progenitor cells in articular cartilage and absence of blood vessels, nerves, and lymphatic vessels. Therefore, the innate self-healing ability of articular cartilage is limited [20]. In contrast to bone regeneration, cartilage regeneration poses significant challenges.



**Figure 1** Graphical illustration of an osteochondral unit structure. Graphical illustration of the zonal structure of chondrocytes, collagen, and the subchondral bone in the osteochondral unit of the knee joint. Please note that this diagrammatic drawing does not represent the actual dimensions. From top-to-bottom, the zones are the SZ-superficial (10–20%), MZ-middle (40–60%), and DZ-deep zones (30–40%).

In summary, chondrocytes lack blood and have limited proliferative potential. Thus, joint damage caused by trauma or disease frequently requires exogenous interventions to stimulate regeneration [21, 22].

## Subchondral bone

Subchondral bone tissue is composed of cortical bone with a dense surface and cancellous bone with distal loose connective tissue and has higher active metabolism than cortical bone [23]. Subchondral bone is well-vascularized and the vascular branches extend into areas of calcified cartilage, which allows for metabolic exchange at the interface between these two tissues and ensures an integrated response to chemical and mechanical stimuli [24]. Calcified cartilage allows for passage of small molecules and is crucial in facilitating biochemical communication between non-calcified cartilage and subchondral bone. The calcified cartilage layer is destroyed in the diseases state and numerous fine fissures within the articular cartilage extend into the subchondral bone. Subchondral bones have blood vessels that reach deep into cartilage [25]. Osteoblasts release chondrolytic substances, such as prostaglandins, leukotrienes, and various (TGF- $\beta$ , BMP, IGF, and FGF) growth factors, during the remodeling of subchondral bone. These substances travel through microcracks and blood vessels from the bone to the cartilage, reaching the articular cartilage [26] and further leading to cartilage loss. Factors released by articular cartilage that promote inflammation and stimulate osteoclast activity may also contribute to deterioration of subchondral bone by enhancing bone remodeling processes [27].

## OC interface

Currently, there is some knowledge regarding the OC interface, which refers to the mineralized region between hyaline cartilage and subchondral bone. This interface is comprised of a layer of calcified cartilage that maintains an efficient junction that is capable of transmitting compressive, tensile, and shear forces from viscoelastic articular cartilage to

mineralised subchondral bone [28]. The OC interface is vital in maintaining the structural stability of the joint and inhibits abnormal mineral deposition, bone growth, and invasion of blood vessels from the underlying bone [29].

OC regeneration has become a major challenge due to the different structural composition, biochemistry, biomechanics, and biological characteristics of cartilage and subchondral bone [30]. However, conventional engineered tissue strategies cannot mimic natural microstructures biologically. To address these issues, a combination of biological elements and biomaterials that can act as scaffolds and drug delivery systems would serve as a promising strategy for repairing OC tissues.

## Signaling pathways related to articular cartilage repair

Cartilage injury repair is regulated by multiple complex signaling pathways, including the transforming growth factor-beta (TGF- $\beta$ ), bone morphogenetic protein (BMP), fibroblast growth factor (FGF), hypoxia-inducible factor (HIF), winged (Wnt)/ $\beta$ -catenin, nuclear factor  $\kappa$ B (NF- $\kappa$ B), mitogen-activated protein kinase (MAPK), and Hedgehog (HH) signaling cascades.

## Growth factor (GF) class signal pathways

GF signaling molecules, including TGF- $\beta$ , BMP, insulin-like growth factor (IGF), and FGF, regulate cellular metabolism and activity during cartilage injury and repair. Specifically, TGF- $\beta$  stimulates chondrocyte proliferation and cartilage matrix synthesis, thereby promoting anabolism and cartilage defect repair. TGF- $\beta$  maintains chondrocyte phenotypic stability and induces proteoglycan and type II collagen production through the Smad2/Smad3 pathway. Conversely, TGF- $\beta$  promotes chondrocyte hypertrophy by stimulating collagen X, MMP-13, osteopontin, alkaline phosphatase, osteocalcin, and vascular endothelial GF expression via Smad1/Smad5/Smad8 signaling. BMP-7 stimulates ECM synthesis in

chondrocytes, inhibits catabolic processes induced by interleukin (IL)-1, IL-6, and fibronectin, and promotes mesenchymal stem cell chondrogenesis [31, 32]. FGF-18 induces chondrocyte proliferation and upregulates SOX-9 (a member of the *Sox* gene family) via fibroblast growth factor receptor 3 (FGFR3) activation, which enhances type II collagen and ECM production [33], while promoting anabolism in human articular chondrocytes. FGF-2 primarily activates FGFR1, upregulates MMP-1 and MMP-13 expression, and enhances ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) production. Notably, ADAMTS inhibits cartilage matrix formation [34] and promotes inflammatory cell infiltration. IGF-1 stimulates chondrocyte proliferation, promotes proteoglycan and type II collagen synthesis, induces mesenchymal stem cell chondrogenesis, and maintains chondrocyte phenotype stability.

### Wnt/ $\beta$ -catenin pathway

The Wnt signaling pathway has a crucial role in regulating proliferation, differentiation, growth, and other cellular functions [35]. The canonical Wnt pathway primarily functions to inhibit cartilage formation and stimulate hypertrophic differentiation in chondrocytes. Specifically, Wnt1, Wnt4, and Wnt8a activate the  $\beta$ -catenin signaling pathway, thereby promoting chondrocyte hypertrophy and endochondral ossification. Inhibiting Wnt/ $\beta$ -catenin signaling alleviates osteoarthritis (OA) and enhances cartilage repair.

### HIF pathway

Chondrocyte survival and functional maintenance depend on adaptive mechanisms mediated by HIF family members. In addition to hypoxia, key cartilage injury factors, including inflammatory cytokines (e.g., IL-1 and tumor necrosis factor- $\alpha$ ) and mechanical compression, also upregulate HIF expression. Studies have shown that HIF has a key role in cartilage injury and repair (specifically, hypoxic overexpression of HIF-1 $\alpha$ ). Hypoxic overexpression of HIF-1 $\alpha$  stimulates chondrogenic differentiation of human bone marrow cells, promotes cartilage formation, and inhibits chondrocyte hypertrophy [36]. HIF-1 $\alpha$  also enhances type II collagen and proteoglycan expression and facilitate cartilage matrix synthesis, thereby mitigating cartilage damage [37].

### HH/smoothed (Smo) pathway

The HH protein family is comprised of Desert (DHH), Sonic (SHH), and Indian Hedgehog (IHH). Among these proteins, IHH signaling critically regulates cartilage formation and chondrocyte proliferation and differentiation in growth plates. Mechanistically, IHH binds its transmembrane receptor (Patched-1 [PTCH1]) and upregulates RUNX-2 expression, the master regulator of chondrocyte hypertrophy, thereby driving chondrocyte hypertrophic differentiation, maturation, endochondral ossification, and ultimately osteophyte formation [38].

### MAPK pathway

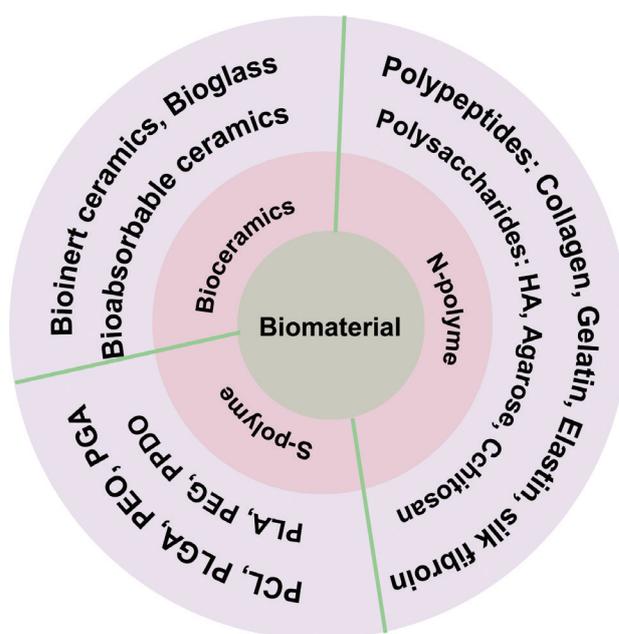
The MAPK signaling pathway regulates essential processes in skeletal development, including cartilage formation, maturation, and endochondral ossification. Dysregulation of MAPK subfamilies, particularly p38 and ERK, have a critical role in chondrocyte dysfunction during OA pathogenesis and progression. Studies have shown that blocking the P38 signaling pathway can inhibit chondrocyte apoptosis in OA cartilage, reduce the expression of inflammatory cytokines (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ), and delay cartilage injury [39].

### NF- $\kappa$ B pathway

NF- $\kappa$ B transcription factors are ubiquitously expressed regulators of inflammation. The NF- $\kappa$ B pathway is activated by multiple stimuli, including fibronectin fragments, pro-inflammatory cytokines (e.g., TNF- $\alpha$  and IL-1 $\beta$ ), mechanical stress, and matrix degradation products during cartilage injury. Activated NF- $\kappa$ B, either independently or through crosstalk with pathways (AMPK and Wnt), suppresses chondrocyte anabolism and stimulates catabolic enzyme secretion. This cascade ultimately drives articular cartilage destruction [40].

## Biomaterials for OC tissue repair and regeneration

A variety of biocompatible materials are currently used in the regeneration of OC tissue, as detailed in **Figure 2**. These biocompatible materials include natural, synthetic, metallic, and inorganic polymers [41]. These substances can be



**Figure 2** Biomaterials used in osteochondral regeneration mainly included natural polymers, synthetic polymers, and bioceramics.

crafted into various forms, such as microparticles, nanoparticles, hydrogels, porous matrices, fibres, and combinations of these materials, to facilitate the delivery of therapeutic drugs.

Polymeric materials are widely utilised to regulate and maintain the release of biological elements, such as bioactive molecules, cells, and genes, that will improve and speed up regeneration of functional bone and/or chondrocytes [42, 43]. Polymer materials are divided into natural and synthetic polymers according to the source. Natural polymers are biocompatible, biodegradable, and bionic and have abundant side groups. Natural polymers are structurally like the ECM and are important in OC regeneration [44, 45]. However, natural polymers have potential immunogenicity and uncontrollable degradation rates [44, 46]. In contrast, synthetic polymers have overcome the above problems and have the advantages of easy fabrication and adjustable physicochemical and mechanical properties [46]. Nevertheless, the primary issues related to synthetic polymers include inadequate cell adhesion as well as possible inflammatory responses caused by acidic degradation byproducts [45, 47].

## Natural polymers

A variety of natural polymers have been used for OC regeneration, including polysaccharides (such as hyaluronic acid [HA] [48, 49], alginate [50, 51], agarose [52, 53], chitosan (CS) [54–56], cellulose derivatives [57]) and proteins (such as collagen [58], gelatin [59, 60], silk fibroin (SF) [61], and fibrin [62]). Natural polymers can facilitate cell interaction, synthesis, and development in tissues due to molecular domains. Collagen, for example, contains the RGD (arginine-Gly-aspartic acid) sequence, which provides extracellular microenvironment signaling that enhances the regenerative process of progenitor cell attachment, migration, proliferation, and differentiation [63]. Gelatin, a derivative of collagen, is a popular natural polymer due to good biodegradability, biocompatibility, biosafety, and cost-effectiveness. Gelatin-based carriers can be used for controlled and continuous delivery of biomolecules to promote the regenerative processes of attachment, migration, proliferation, and differentiation of mesenchymal stem cells (MSCs) [64]. CS polymers share structural similarities with two major cartilage molecules (HA and glycosaminoglycan molecules), which have successfully achieved subchondral bone and cartilage regeneration [65, 66]. Silk polymers obtained from silkworm cocoons or spider silk are also widely used for OC tissue regeneration [67]. Silk polymers can be mixed with synthetic polymers to enhance mechanical stability and make synthetic polymers easier to customize and process [68]. The mechanical properties of silk fibroin combined with nano-CaP are better than pure silk fibroin alone [69].

## Synthetic polymers

At present, the commonly used synthetic polymers in OC tissue regeneration include polycaprolactone (PCL), polylactic acid-glycolic acid (PLGA), polyethylene oxide (PEO), polyglycolic acid, polylactic acid (PLA), polyethylene

glycol (PEG), polycyclohexanone, and polypropyl fumarate [41, 70]. These polymers are hydrophobic and have rigidity, elasticity and porosity [71], which can be manipulated to achieve appropriate mechanical behaviour based on the properties of the material. Hydrophilicity can be improved by mixing hydrophobic and hydrophilic polymers, which facilitate cell fixation [41, 72, 73]. As mentioned earlier, the main advantages of using synthetic polymers are complete control of the structure and better mechanical properties. Synthetic polymers are also free of disease transmission or polymer supply constraints. However, biocompatibility can become a major concern when matrices made from synthetic polymers are used. In addition, synthetic polymers are prone to degradation and promote inflammation. PLA and PLGA offer the benefit of prolonged release of encapsulated substances over an extended duration. Nevertheless, generation of acidic degradation byproducts causes an inflammatory tissue reaction and leads to denaturation of bioactive factors. Therefore, synthetic polymers have been combined with natural polymers as delivery systems to achieve better biocompatibility [74, 75]. Neutralization between chitosan and PLGA degradation products could be achieved by wrapping chitosan/PLGA microspheres on the surface of BMP-2 [76]. Some studies have shown that PLLA can promote chondrocyte differentiation when combined with natural polymers, such as chitosan and silk, suggesting that delivery systems combining natural and artificial polymers have superior ability to promote OC regeneration [77, 78].

## Metal materials

Titanium, titanium alloy, cobalt-chromium alloy, and stainless steel are commonly used metal materials in orthopedics. Metallic materials are characterized by excellent mechanical properties and biological inertness. Titanium and titanium alloys are the predominant metallic materials utilized in orthopedic application due to excellent compatibility and integration with bone tissue [79, 80]. By the mid-1980s, the focus of research on metallic materials began to shift from inert to bioactive and biodegradability. In recent years, magnesium-, strontium-, and zinc-based alloys have been successfully applied to orthopedic implants due to excellent mechanical properties and good bioactivity and biodegradability [81–84]. The combination of metallic materials and bioceramics, such as calcium phosphate, tricalcium phosphate (TCP), and HA, can enhance mechanical properties and promote bone mineralization, further aiding the fixation of implants with subchondral bone [85]. However, metallic materials are limited by inappropriate degradation rates, corrosion, and release of wear particles.

## Inorganic materials

Bioceramics and bioapatites in bone have similar physicochemical properties and therefore have similar properties in terms of biodegradability, osteoconductivity, and osteoinducibility. Bioceramics promote bone regeneration by solubilizing and reprecipitating and combining with other ions

in the tissue microenvironment to form carbonate apatite, which induces protein adsorption and enables cell attachment and differentiation [86]. Because TCP is more soluble than hydroxyapatite (HAP), the ratio of TCP-to-HAP can be adjusted to adjust the absorption rate of the material and match the rate of bone formation in biphasic biocrystals [41, 71, 87]. Bioceramics include bioinert ceramics, bioabsorbable ceramics, and bioactive glasses. Bioinert ceramics, like alumina and zirconia, can provide sufficient mechanical strength for the regeneration of subchondral bone but bioinert ceramics lack substantial biological interactions with the surrounding tissue. Bioresorbable ceramics, including calcium phosphate, calcium carbonate, calcium phosphate cements, and calcium silicate, can be gradually absorbed and replaced by new bone tissue *in vivo* [86]. Bioactive glasses (BGs), including silicate, phosphate, or borate based glasses, can be directly combined with surrounding tissues to form adhesive osteogenesis [88]. The excellent bioactivity of BGs can be attributed to the following: i) the interaction between collagen fibres in the vicinity and bone surface, which makes the collagen fibres tightly bound to the bone; and ii) the gradual dissolution and re-precipitation in physiologic fluids result in the formation of hydroxycarbonate apatites, which endow hydroxycarbonate apatites with bone conduction properties [89, 90]. The release of ions, especially cations, is also of great significance for OC regeneration. For example, incorporation of  $\text{Cu}^{2+}$  into BG scaffolds greatly enhance cartilage regeneration and OC interface repair, induce the transformation of macrophages to an anti-inflammatory phenotype, and suppress the inflammatory response of OC tissue [91]. BGs are frequently utilized in bone tissue engineering. BGs are resistant to chemical corrosion and wear, which can stimulate bone tissue formation [92–94].

In addition, bioceramics are easy to produce, inexpensive, and safe, making bioceramics widely used. In fact, bioceramics have been used in up to 53% of material applications for subchondral bone regeneration. However, bioceramics also have drawbacks, including limited flexibility, a high tendency to break, excessive rigidity, and inadequate resistance to fractures. Therefore, the development of biomaterials that combine the advantages of bioactive ceramics with polymers to obtain more ductile, tractable, and controllable properties, including stiffness, fracture toughness, porosity, and surface topography, for OC regeneration is a new research direction. For example, the combination of natural and synthetic polymers, such as gelatin, collagen, silk fibroin-based hydrogels and PLA with bioceramics, has resulted in improved mechanical properties and better osteoinduction [95]. Advances in this area have important implications for OC regeneration because improved healing of the subchondral bone leads to better results in cartilage repair.

## Analysis of clinical settings

Natural polymers offer significant advantages as drug delivery carriers, including high biocompatibility, biodegradability, and low immunogenicity. These materials enhance cell

migration, proliferation, and differentiation, while the specific molecular domains provide structural and functional guidance for cells at various developmental stages. However, natural polymers typically exhibit poor mechanical strength, low stiffness, and higher production costs [96]. Synthetic polymers offer superior mechanical strength, enhanced elasticity, low cytotoxicity, and tunable degradation kinetics compared to natural polymers. The design flexibility enables fabrication into patient-specific architectures with tailored porosity and dimensions that accommodate cell migration dynamics and tissue regeneration requirements [97, 98]. Furthermore, significant advances in electrospinning and 3D printing technologies have enabled faster fabrication of polymer-based carriers. Despite these manufacturing advantages, the inherent lack of biological activity limits interactions with host tissues. Such carriers typically exhibit hydrophobic surfaces that impair cell adhesion and proliferation. To address this finding, researchers have enhanced hydrophilicity and introduced bioactive structures through polymer surface functionalization, especially using plasma coating techniques.

Metal materials are particularly suitable for orthopedic implants addressing subchondral bone defects due to the high load-bearing capacity. However, the non-biodegradability and potential for wear particle generation restrict broader clinical applications. Recent studies have revealed that magnesium and magnesium alloys offer promising alternatives, combining excellent biodegradability and biocompatibility with osteogenic properties. Nevertheless, rapid degradation in the early post-implantation phase compromises mechanical integrity, which hinders utility [99, 100].

Ceramics exhibit high hardness, excellent wear resistance, and favorable biocompatibility. Ceramic implants generate significantly fewer wear particles compared to metallic and polymer-based implants. Nanoceramic composites feature smaller grain sizes than microceramics, enhancing the overall mechanical performance, tribological behavior, and biological functionality *in vivo*. Bioinert ceramics offer advantages, including support for tissue regeneration, biocompatibility, and improved mechanical strength. However, bioinert ceramics have unstable crack propagation, hydrothermal aging, and high manufacturing costs. Conversely, bioactive ceramics promote direct bone bonding (osteoconduction) and demonstrate high chemical stability but the inherent brittleness and complex processing limit clinical adoption. The advantages and disadvantages of the biomaterials are summarized in **Table 1**.

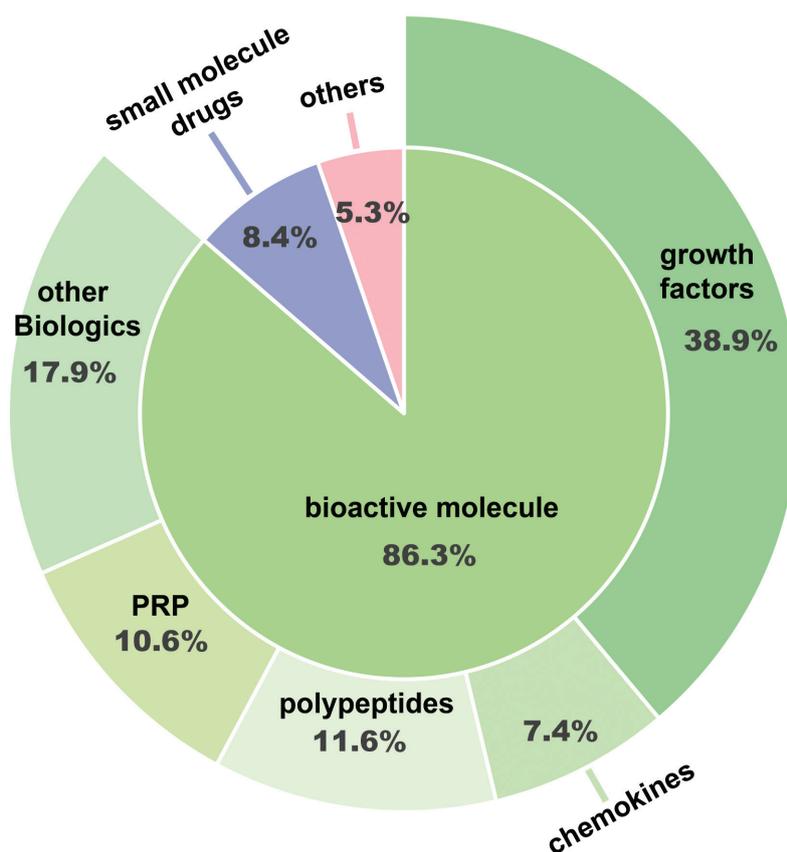
## Biological cues for OC tissue regeneration drugs

In recent years the therapeutic strategy of combining drugs with biomaterials has received extensive attention in addition to the necessary surgical treatment. These drugs mainly include a series of bioactive molecules, small molecule drugs, and other drugs, as shown in **Figure 3**.

**Table 1** Common biomaterial types used in osteochondral repair and regeneration: advantages and drawbacks

Type	Name	Advantages	Drawbacks
Natural polymer	HA	Chemically modifiable, natural GAG in cartilage matrix	Low mechanical strength, requires modification to form 3D structures
	Alginate	Cytocompatibility and regulated gelation	Low mechanical stability
	Chitosan	Biocompatible, non-cytotoxic, contains cartilage components	Slow gelation for <i>in situ</i> applications
	Collagen	Excellent biocompatibility, bioresorbable, and natural component of ECM; already used clinically	Immunogenic, poor mechanical strength, expensive
	Fibrin	Excellent mechanical properties and high versatility	High shrinking ability, increased degradation rate, and less mechanical stability
Synthetic polymers	PLA	Easily processed, elongated degradation, high strength	Acidic byproducts, auto-catalytic degradation
	PLGA	Selectable degradation based on co-polymer ratio	Acidic byproducts, poor long-term stability
	PEG	Easily functionalized	Non-degradability, no inherent biologic impact
	PCL	Great mechanical strength, elasticity	Hydrophobicity, slow degradation, intracellular resorption
Metal Materials	Titanium and its alloys	Excellent mechanical properties, biological inertia	Non-degradability, tiredness, ion leakage, and infection risk
	Magnesium/strontium/zinc-based alloys	Excellent mechanical properties, good biological activity, biodegradability	Inappropriate degradation rate, corrosion, release of wear particles
Inorganic materials	Bioceramics	Promote bone regeneration and control biodegradation	Fragile, do not resist mechanical stresses

HA: hyaluronic acid; PLA: polylactic acid; PLGA: poly (lactic-co-glycolic acid); PEG: polyethylene glycol; PCL: polycaprolactone.

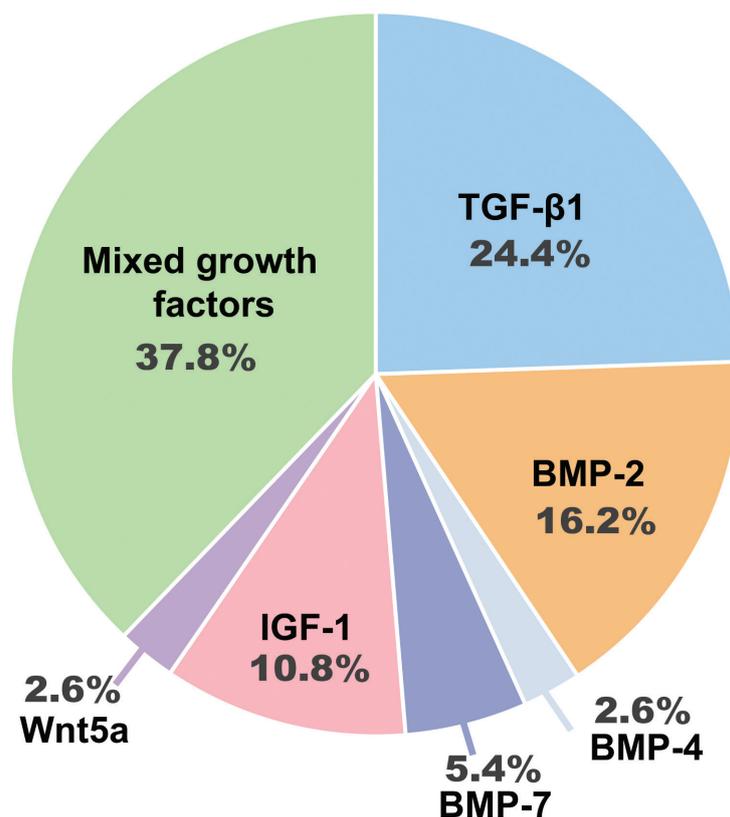


**Figure 3** Drugs used to promote osteochondral regeneration are mainly bioactive molecules, small molecule drugs, and other types of drugs. Among the bioactive molecules were growth factors (GFs), platelet-rich plasma (PRP), chemokines, peptides, and other types of biological agents (the systems reported since 2015).

## GFs

GFs promote the regeneration of new tissues by regulating the synthesis of cellular proteins and interacting with specific

transmembrane receptors. GFs stimulate cellular signaling pathways that trigger cell differentiation, growth, proliferation, adhesion, survival, and migration upon binding to GF receptors. A variety of GFs have been used for OC tissue



**Figure 4** Analysis of growth factors for osteochondral regeneration, including transforming growth factor-β1 (TGF-β1), bone morphogenetic protein (BMP)-2, -4, and -7, insulin-like growth factor-1 (IGF-1), wingless family member 5a (Wnt5a), mixed growth factors, or other types of growth factors (the systems reported since 2015) [214].

regeneration, as shown in **Figure 4**. GFs accelerate healing and regeneration of OC tissue through various methods, such as regulation of metalloproteinases and inflammatory markers, recruiting cells, and remodeling and synthesizing the ECM. In particular, the TGF-β superfamily members have a key role in the development of bone and cartilage [101]. TGF-β1 and TGF-β3 have been shown to have effects on cell proliferation and differentiation into osteoblasts or chondrocytes that depend on timing, dosage, and environmental factors [102]. Expression of TGF-β, especially TGF-β1 and TGF-β3, is significantly increased in injured chondrocytes and the efficiency of TGF-β signaling decreases with age [103]. Activated TGF-β1 and TGF-β3 can effectively stimulate the synthesis of aggrecan and type II collagen in chondrocytes and can induce MSCs to differentiate into chondroblasts *in vitro*. TGF-β1 can induce MSCs to differentiate into ectopic cartilage to repair cartilage defects *in vivo* [104]. BMPs are members of the TGF-β superfamily of secreted ligands. BMPs can induce the differentiation of MSCs into chondrocytes and osteoblasts and have an important role in the development and formation of bone and cartilage [105]. Specifically, BMP-2, -4, and -7 have been used clinically for bone regeneration. For example, the combined administration of BMP-7/BMP-2 improved healing in a rabbit cartilage injury model. BMP-4-expressing myogenic stem cells enhance chondrogenesis and significantly improved articular cartilage repair in a rat model [104].

TGF-β and BMPs act on a tetrameric receptor complex consisting of two TGF-β type I receptors (TβRI/ALK5) and

two type II kinase receptors (TβRII). The tetrameric receptor complex regulates mesenchymal stem cell (MSC) differentiation during bone development, bone formation, and bone homeostasis by regulating classical Smad-dependent (TGF-β/BMP ligands, receptors, and Smads) and non-classical Smad-dependent signaling pathways (p38 mitogen-activated protein kinase/p38 MAPK) [106]. In addition to a role in bone development, TGF-β and BMPs regulate postnatal bone and cartilage maintenance. TGF-βs have an important role in bone construction by osteoblasts and bone destruction by osteoclasts through osteoclast-mediated ATP6I-specific extracellular acidification [107] and cathepsin K-specific ECM proteins [108].

IGF-1 and BMP-2 have been encapsulated within gelatin microparticles and incorporated into a high molecular oligomer [poly (ethylene glycol) fumarate] (OPF) hydrogel matrix with precise spatial distribution. The OPF hydrogel matrix promotes subchondral bone formation in a rabbit model of OC injury. Although these dual delivery systems do not improve cartilage repair, the dual delivery systems do show the potential of spatially-directed, multiple GF delivery vehicles for OC tissue repair [109]. Kim et al. [110] constructed a dual hydrogel delivery system consisting of TGF-β3 and IGF-1 to promote OC tissue regeneration in a full-thickness OC defect model in rabbits. A novel nanoparticle that can deliver SDF-1 and BMP-2 simultaneously with high biomolecular loading capacity and prolonged release effect can stimulate MSC migration and promote better OC regeneration. Shi et al. constructed an injectable

hydrogel by loading nanosilicate (SN) and stromal cell-derived factor-1 alpha (SDF-1 $\alpha$ ) into gelatin-methacryloyl (GelMA). The hydrogel effectively promotes the migration and homing of MSCs and has efficient OC repair ability. GelMA has shown good bone regeneration ability in a rat calvarial defect model [111].

## Small-molecule drugs

In addition to GFs, much attention has been paid to the potential of small molecule drugs in cartilage repair and regeneration. These small molecules primarily target signaling pathways, enzymes, and receptors that have roles in the formation and upkeep of cartilage. One of the primary mechanisms through which small molecules facilitate the repair and regeneration of cartilage is by stimulating and inducing chondrogenesis. Kartogenin (KGN), a heterocyclic small molecule, can enhance the chondrogenic differentiation of MSCs by upregulating chondrogenic gene expression and characteristic chondrocyte activity. KGN has shown a cartilage regeneration effect in a mouse model [112]. It has been reported that KGN promotes cartilage repair and prevents cartilage degeneration and subchondral bone degradation through the TGF $\beta$ /Smad signaling pathway. KGN promotes the formation of a cartilage-like transition zone at the tendon-bone junction and stimulates collagen synthesis [113]. KGN can induce bone marrow stem cells (BMSCs) to differentiate into chondrocytes. *In vivo* studies have demonstrated the potential cell homing effect of KGN-loaded nanoparticles to recruit endogenous BMSCs and synovial mesenchymal stem cells (SMSCs) [114]. KGN has been increasingly used to promote OC regeneration. For example, a HA hydrogel (m-HA) loaded with KGN PLGA nanoparticles (m-HA+KGN treatment) achieve favorable cartilage repair in a rabbit model [48]. KGN can improve the chondrogenic capacity of human bone marrow stem cells (hBMSCs) *in vitro* and *in vivo* and effectively supports the regeneration of hyaline cartilage and subchondral bone in a rat model of OC defects [115]. A bilayer scaffold combined with KGN and human stem cell-specific binding oligonucleotide (aptamer A19S) specifically recognizes and binds to MSCs. By recruiting MSCs to the scaffold, the bilayer scaffold enriched MSCs around OC defects and stimulated MSC directional differentiation. The excellent cartilage and subchondral bone repair ability of the bilayer scaffold was demonstrated by a rat OC defect model [50].

Small-molecule drugs can also selectively modulate the subcellular localisation and downstream transcriptional activity of transcription factors. KGN attaches to filamin A, disturbing the filamin A connection with the transcription factor core binding factor beta subunit (CBF $\beta$ ) and prompting chondrogenesis by controlling the CBF $\beta$ -Runx1 transcription program [116]. In addition, subchondral bone is highly vascularized and tiny branches of the subchondral bone blood vessels can extend into areas of calcified cartilage. This feature allows for metabolic exchange between the two tissues and an integrated response to chemical and mechanical stimuli. Therefore, promoting angiogenesis is crucial for cartilage repair and regeneration. Small molecules, such as

vascular endothelial growth factor A (VEGF) and FGF, can promote the growth of new blood vessels and help cartilage repair and regeneration. VEGF is a chondrocyte survival factor during development and is essential for bone formation and bone growth. Increased VEGF expression is associated with catabolic processes in chondrocytes and synoviocytes [117]. It should be noted that although small molecules have demonstrated encouraging outcomes in fostering cartilage repair and regeneration in preclinical studies, additional clinical studies are needed to validate safety and efficacy, as well as to assess long-term efficacy [118].

Although the addition of a single bioactive factor in the delivery system holds promise, the continuous proliferation and differentiation of OC chondrocytes is a complex process involving multiple factors. Therefore, to further improve the tissue regeneration effect, multi-factor combination therapy is necessary.

## Anti-inflammatory drugs

Inhibition of inflammation is also a crucial role in fostering cartilage repair and regeneration. Inflammation can lead to cartilage damage and impede the repair process. The use of anti-inflammatory drugs is primarily designed to reduce inflammation and/or pain. Commonly used drugs include acetaminophen, NSAIDs, opioids, and/or glucocorticoids [119]. NSAIDs are the most frequently used drugs for the treatment of articular cartilage damage. NSAIDs inhibit the cyclooxygenase enzymes, COX-1 and COX-2, which convert arachidonic acid into prostaglandin E<sub>2</sub>, which are the precursors of prostaglandins and prostacyclins that cause inflammation and pain, respectively [120, 121]. However, prolonged use of NSAIDs may adversely affect cartilage repair. NSAIDs hinder the proliferation and differentiation of chondrocytes responsible for cartilage repair and potentially delay healing [122]. Therefore, NSAID administration should be restricted to the acute phase of an injury and used cautiously in chronic cases. In addition, studies suggest that small molecules, such as celecoxib and curcumin, can mitigate inflammation and enhance cartilage repair and regeneration by suppressing the production of inflammatory mediators, such as prostaglandins and TNF- $\alpha$  [123].

## Immunomodulators

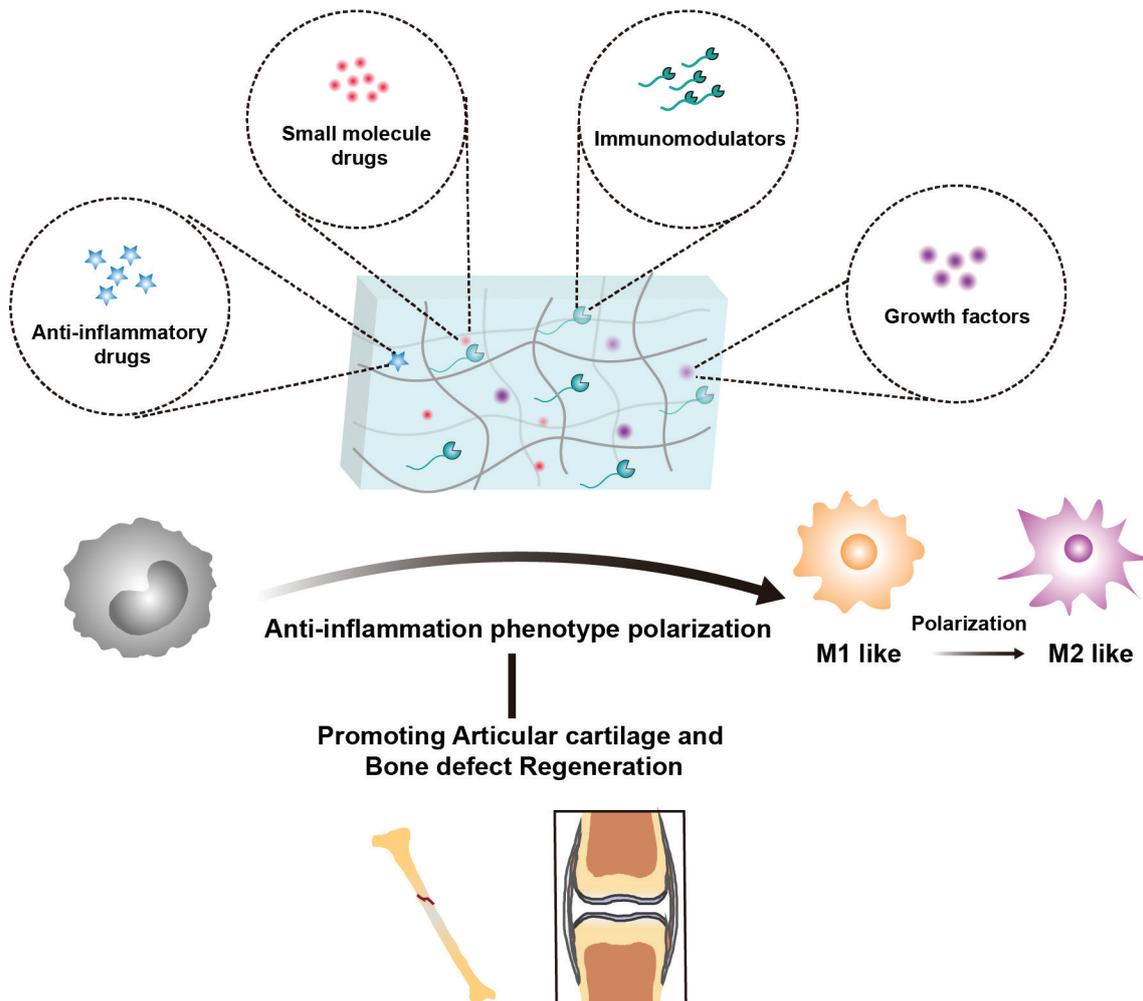
The immune microenvironment is pivotal in the regeneration of bone, cartilage, and soft tissue, especially macrophages. Dysregulated activation of macrophages can impede the process of OC tissue regeneration. Macrophages exist in various phenotypes, including the M0 (resting state), M1, and M2 phenotypes. During the initial phase of tissue repair, macrophages predominantly polarize toward the M1 phenotype and release pro-inflammatory cytokines, including TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . This response promotes inflammation while facilitating the elimination of pathogens, senescent cells, and necrotic debris. In the mid-to-late stages of inflammation, macrophages undergo polarization toward the M2 phenotype and secrete anti-inflammatory cytokines, such as IL-4 and

IL-10. This transition facilitates the resolution of inflammation and actively promotes tissue regeneration through matrix remodeling and angiogenesis [124, 125]. The macrophage phenotype is critical in OC repair [126]. OC tissue regeneration can be improved by regulating the polarization of macrophages and promoting the transition from M1-to-M2 macrophages, as shown in **Figure 5**. Chitosan engages macrophage receptors (CR3/TLR4), which down-regulates the MAPK/ERK signaling cascade. This process inhibits NF-κB activation and subsequently suppresses expression of pro-inflammatory mediators, including TNF-α, IL-6, and inducible nitric oxide synthase (iNOS) [127]. Loading blueberry anthocyanins into injectable hydrogels composed of carboxymethyl chitosan (CMC) and HA mediates NF-κB downregulation, promotes polarization of M1 macrophages toward the M2 phenotype, and enhances tissue repair mechanisms [128]. HA engages TLR2 and TLR4 receptors on macrophages, potentiating pro-inflammatory responses through upregulated expression of TNF-α and IL family factors (e.g., IL-1β and IL-6) [129]. A platelet-rich plasma (PRP)-GelMA hydrogel scaffold has successfully polarized M1 macrophages into M2 macrophages by immunomagnetic modulation of the local microenvironment of OC tissue. The PRP-GelMA hydrogel scaffold was shown to promote OC repair in a rabbit model [130].

### Other biological cues

An HA-based microcryogel can induce differentiation of MSCs into cartilage and osteogenesis and has the ability to self-assemble into OC organoids. Implantation of the gel into OC defects in dogs was shown to effectively promote the regeneration of articular cartilage and subchondral bone [131]. The results of a meta-analysis involving 21,163 randomized participants demonstrated significant advantages of HA in the treatment of knee injuries [132].

Oxidative stress caused by high levels of reactive oxygen species (ROS) in the local microenvironment can destroy the repair process following OC injury, which is an adverse factor for poor tissue repair. Evidence suggests that age-related chondrocyte damage is associated with an imbalance of ROS, which are involved in cartilage degradation and chondrocyte death. Overexpression of antioxidant genes in mice reduces joint damage [133]. A nanoenzyme hydrogel effectively alleviated the symptoms of cartilage injury, including suppression of local inflammatory factors and improved osseointegration, by scavenging endogenous overexpressed ROS. These results suggested that reducing oxidative stress is an effective treatment that promotes OC repair [134]. Researchers have designed a hydrogel drug delivery system that combines polyphenol-based SF and the



**Figure 5** Schematic diagram of the regulation of macrophage phenotypes by drug delivery systems in osteochondral repair.

antioxidant, tannic acid (TA), which can eliminate ROS in the local microenvironment, promote BMSC proliferation, and combat oxidative stress. The hydrogel drug delivery system provides a suitable microenvironment for OC regeneration [135]. The combination of biomaterials and bioactive molecules to clear endogenous ROS and provide a suitable local microenvironment for tissue regeneration has become a promising approach for the treatment of OC defects.

Glucosamine is the basic substance for the synthesis of aminoglycans and is a natural component of articular cartilage. Glucosamine decreases with age, which leads to continuous degeneration and wear of articular cartilage. Chondroitin sulfate is a type of glycosaminoglycan that synthesizes proteoglycans and protects chondrocytes. Glucosamine may alleviate pain caused by impaired lubrication function and reduced HA concentration. Glucosamine may alleviate pain caused by impaired lubrication function and reduced HA concentration [136]. Combined chondroitin sulfate-glucosamine therapy reduces serum biomarkers associated with cartilage and bone degradation, prevents cartilage degeneration, and decreases levels of inflammatory mediators, such as IL-1 $\beta$  and TNF- $\alpha$  [137].

## OC tissue regenerative drug delivery system

With the continuous development of materials science, a new strategy for OC tissue regeneration is to combine a variety of composite materials to construct an ideal functional delivery system (Table 2), simulate the tissue microenvironment, and achieve the continuous release of bioactive molecules with different spatial and temporal distributions.

## Hydrogel scaffold delivery system

Hydrogels are densely water-filled polymer networks that are simple to create, capable of encapsulating molecules within a hydrated structure, and have been thoroughly researched for regenerating cartilage tissue. The main applications of hydrogels are cellular and bioactive substance delivery systems. The hydrogel delivery system can provide support for chondrocytes and the ECM due to the nature of the hydrogel and the OC organization [138]. The distinctive structure of the hydrogel creates an optimal local microenvironment that supports the chondrogenic differentiation of MSCs and enhances cartilage regeneration. Osteoinduction refers to the ability to stimulate stem and progenitor cells to differentiate into osteogenic lineages in a laboratory setting [139]. Chondrogenesis refers to the process by which MSCs differentiate into chondrocytes [140]. OC regeneration is associated with the generation of cells associated with bone and cartilage. A CS-based hydrogel delivery system has enhanced cartilage regeneration and greatly improved repair of cartilage defects by delivering MSCs and improving the regeneration microenvironment [141]. The ideal hydrogel delivery system should have a microenvironment conducive to MSC adhesion and proliferation, and provide inductive signals to promote osteogenic and chondrogenic differentiation of MSCs.

The immunomodulatory hydrogel synthesized by combining the advantages of a hydrogel and M2 macrophages has a very good effect on OC regeneration. Ji et al. [105] developed a macrophage regulatory drug delivery system composed of porous chitosan (CS) microspheres and hydroxypropyl chitin (HPCH) hydrogel, in which dimethyloxyallyl glycine (DMOG) was encapsulated in a thermosensitive HPCH hydrogel (HD). KGN was coupled to porous CS microspheres (CSK-PMS). The prepared

**Table 2** Bioactive molecule delivery systems currently used in osteochondral regeneration

Bioactive molecule	Delivery system	Application
TGF- $\beta$ 1	PLGA nanoparticle	Increased MSCs adhesion and proliferation; increased cartilage differentiation [156]
TGF- $\beta$ 1/IGF-I	PLGA microspheres	Reduced initial burst sustained release [169]
TGF- $\beta$ 3/IGF-I	Gelatin Microspheres Hydrogel systems	Promoted osteochondral tissue regeneration [110]
BMP-2	Alginate PLGA microspheres Hyaluronic hydrogel system	Sustained release Promoting bone repair [164, 165]
BMP-7/TGF- $\beta$ 2	Nanoparticle	Cartilage regeneration BMSCs chondrogenic differentiation [155]
BMP-2/bFGF	Gelatin nanoparticles	Programmed and sustained release [84]
BMP-2/TGF- $\beta$ 3		Promoted bone formation [182]
BMP-2/IGF-I	Gelatin particles Hydrogel system	Promotes the subchondral bone formation [109]
BMP-2/VEGF	HAp Hydrogel system	Promoted osteogenesis and angiogenesis [187] controlled and sustained release [190]
bFGF/ HAp	Hydrogel system	Promoted hyaline cartilage and subchondral bone formation [192]

TGF: transforming growth factor; BMP: bone morphogenetic protein; PLGA: polylactide-co-glycolide; VEGF: vascular endothelial growth factor; BMSCs: bone marrow mesenchymal stem cells; IGF-I: human insulin-like growth factor I; HAp: hydroxyapatite.

HD/CSK-PMS composite hydrogel delivery system can effectively regulate the microenvironment at the defect site, achieve M2 polarization of local macrophages, and promote cartilage regeneration. Rapidly degrading HD is conducive to the regeneration of hyaline cartilage, while highly stable CSK-PMS supports the regeneration of endochondral osteogenesis and subchondral bone. *In vitro* and *in vivo* evaluations have shown that the newly developed HD/CSK-PMS, as a controlled drug delivery system, can effectively regulate the microenvironment at OC defects, polarize local pro-inflammatory M1 macrophages into pro-regenerative M2 macrophages, and promote cartilage regeneration. Yang et al. [142] designed a cell adhesion peptide (CRGD)-modified PEG hydrogel to improve the ability to promote chondrogenic differentiation by increasing the number of MSCs and inducing macrophage polarization to the M2 phenotype. IL-4 enhances the immunoregulatory capacity of macrophages by promoting anti-inflammatory polarization. IL-4 suppresses pro-inflammatory cytokine expression within OC injury microenvironments, including IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 in synovial fibroblasts and chondrocytes, thereby mitigating tissue-destructive inflammation [143]. Incorporating anti-inflammatory bioactive factors (e.g., IL-4 and anthocyanins) into hydrogel systems attenuates pathologic inflammation and orchestrates pro-regenerative microenvironments through targeted immunomodulation and enhanced cellular cross-talk. Gong et al. [59] designed a hydrogel delivery system containing IL-4, which comprises an IL-4-loaded GelMA upper layer and a porous polycaprolactone and hydroxyapatite (PCL-HAP) lower layer. Sixteen weeks after implantation of the hydrogel delivery system containing IL-4 in a rabbit OC defect repair model, the histologic score of the IL-4-loaded hydrogel delivery system was 24 $\pm$ 2, which was 2.18 times higher than the untreated group and 1.5 times higher than the unloaded IL-4 group. These findings indicated that the hydrogel delivery system loaded with IL-4 promotes the regeneration of cartilage and subchondral bone and increases the formation of new cartilage and bone tissue. Zhao et al. [144] modified heparin with maleimide and combined the heparin modification with IL-4 to prepare the PEG-SH hydrogel. The hydrogel loaded with IL-4 has good biological properties. Overexpression of M2 macrophage-related phenotypic genes polarizes M2 in macrophages and regulates the immune microenvironment. The effect of co-culture with MC3T3-E1 cells in promoting osteogenesis is shown in **Figure 6**.

The physicochemical properties of the hydrogel delivery system can be customized depending on the choice of material. Zhang et al. [145] designed an enzyme-linked cross-linked SF-laponite (LAP) nanocomposite hydrogel. Incorporation of a small amount of LAP (1% w/v) into SF greatly improved the mechanical properties and hydrophilicity of the hydrogel and promoted the osteogenic and chondrogenic differentiation of BMSCs. SF-LAP hydrogel promoted simultaneous regeneration of cartilage and subchondral bone in a rabbit full-thickness OC defect model. In addition, Zhu et al. [146] added bacterial cellulose to alginate dual network (DN) hydrogel and greatly improved the biological strength

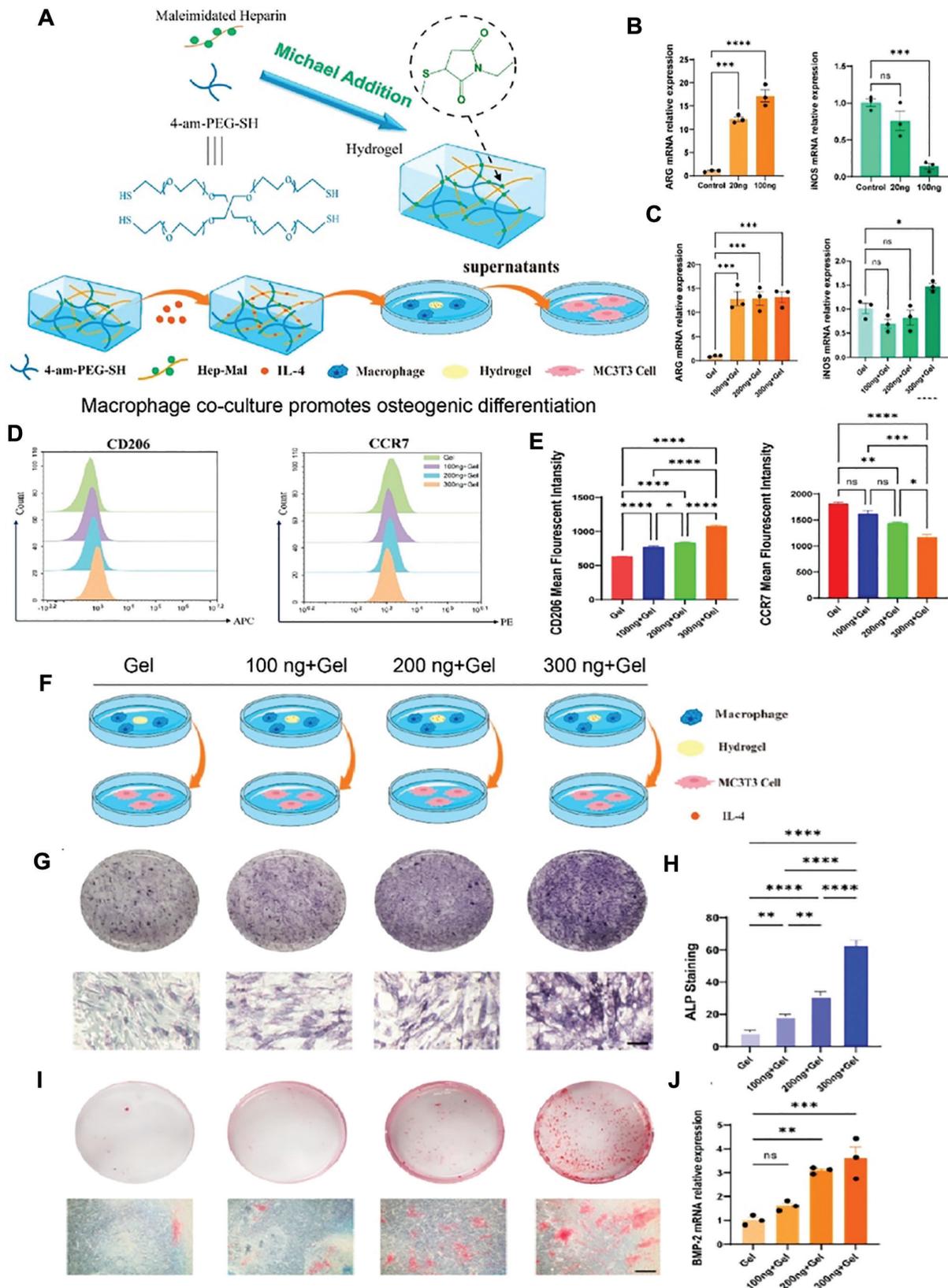
of the hydrogel. Two different sizes of HAP, HAP particles, and HAP nanocrystles, were incorporated into the hydrogel to promote the simultaneous repair of cartilage and subchondral bone in the rabbit OC defect model.

In addition, hydrogel delivery can also be tailored according to pH or temperature changes in the local OC microenvironment to control the release of bioactive substances [147]. The continuous release of bioactive substances can provide a continuous chemical signal for tissue regeneration. George et al. [148] designed a pH-responsive double crosslinked hydrogel by combining a crosslinked catechol functional group (DOPA) and OPF. The incorporation of DOPA alters the surface chemistry and mechanical properties of the hydrogel. The attachment and proliferation of pre-osteoblasts were enhanced. Nelson et al. [149] designed an injectable, degradable, sustained-release, and sequential release thermos-responsive hydrogel. This hydrogel prolonged the continuous release of bovine serum albumin (BSA).

While hydrogels offer sustained drug release and chemical signaling capabilities, hydrogels specifically tailored for osteoinduction and chondrogenesis have gained popularity in OC regeneration. However, using bioactive molecules or materials for osteoinduction and chondrogenesis frequently poses challenges, including instability, immunogenicity, high cost, and potential clinical side effects, all of which require careful consideration. Second, due to inherently weak mechanical properties, hydrogels used alone are more suitable to provide injectable and local OC regeneration therapy. Hydrogels are combined with synthetic polymers to improve structural and physical properties to further enhance bone and cartilage regeneration.

## Nanoparticle delivery system

Nanomaterials have been developed for the regulation of drug release site and timing due to excellent controlled release properties. Nanocarriers are versatile with respect to size, shape (spherical, rod-shaped, or cuboidal), composition, and surface charge. In addition, the incorporation of nanomaterials can reduce the side effects of irritating and sensitive drugs. Nanomaterials are used as effective drug delivery systems to promote cartilage and bone tissue regeneration based on the advantages of nanomaterials as delivery systems. Frequently used degradable nanomaterials include collagen, CS, HAP, PLA, and PLGA; non-degradable nanomaterials include lipids, dendrimer, silica, and metals [150, 151]. A biphasic semi-interpenetrating polymer network (SIPN) hydrogel containing CS nanoparticles for functional cartilage repair [152]. Radhakrishnan et al. [151] designed a hydrogel loaded with CS nanoparticles (CS-NPs) and nano-hydroxyapatite (nHAP) to successfully promote cartilage regeneration and subchondral bone formation. Oliveira et al. [153] showed that dexamethasone-loaded carboxymethyl CS/poly (amido-amine) dendrimer nanoparticles (CMCh/PAMAM) can be used as intracellular nanocarriers. In the absence of typical osteogenic inducers, dexamethasone (Dex) can be used to control the osteogenic differentiation of stem cells *in vivo* and stimulate new bone formation. Additionally, GFs and target proteins can also promote OC regeneration through



nanoparticle delivery systems. Previous studies have shown that nanoparticles loaded with GFs, such as BMP-2, BMP-7, and TGF- $\beta$ , have the potential to promote cartilage regeneration [154]. Lim et al. [155] designed a nanodelivery system loaded with dual growth factors (BMP-7/TGF- $\beta$ 2) to control the rate of GF release, which is an ideal delivery system for cartilage regeneration and chondrogenic differentiation of MSCs. Castro et al. [156] reported that PLGA nanospheres loaded with TGF- $\beta$ 1 could increase MSCs adhesion, proliferation, and OC differentiation *in vitro*.

Gelatin, a derivative of collagen, has been widely used to encapsulate bioactive molecules. Injectable colloidal gels fabricated from countercharged gelatin nanospheres have shown great potential for bone fillers and programmed drug delivery due to good clinical manipulation, ease of functionalization, and cost-effectiveness [157]. Gelatin gels overcome the disadvantages of PLGA colloidal gels, such as the lack of cell attachment sites and the release of acidic degradation products, and show obvious advantages in the programmed delivery of bioactive molecules [158]. Wang et al. [159] obtained a continuous release delivery system characterized by rapid bFGF release and durable BMP-2 release by loading bFGF onto cationic nanospheres with low cross-link density and BMP-2 onto anionic nanospheres with high cross-link density. Gelatin nanoparticles have been demonstrated to be suitable carriers for programmed and sustained release of a variety of therapeutic proteins for tissue regeneration.

Metal nanoparticles have been widely used in tissue healing and regeneration because of the intrinsic chemical, physical, and biological properties, such as the release of bioactive ions (Ca, Mg, Sr, Si, B, Fe, Cu, and Zn), inducing changes in cell phenotype or regulating the immune microenvironment. Metal nanoparticles can affect stem cell proliferation and differentiation by regulating signaling pathways, ROS production, and multiple transcription factors [160]. The biophysical properties of metal nanoparticles, such as morphology, charge, size, electrostatic interactions, and stiffness, can be modulated by binding to polymers [84]. For example, when metal particles are combined with porous poly(DL-lactide) [PPLA], the bioactive interface provided by metal particles compensates for the lack of bioactive cell-material interface and explosive release of GFs in PPLA, resulting in sustained release of BMP-2 combined with the osteogenic ability of metal particles. The migration and osteogenic differentiation of BMSCs were successfully promoted (Figure 7) [161].

## Microsphere delivery system

Microparticles are more advantageous in the application of controlled drug delivery for OC regeneration than drug-loaded nanomaterials due to side effects, such as early burst release, high chemical toxicity, and short retention time [162]. Therefore, polymeric microparticles have been widely used for the delivery of cells and bioactive substances. Among the polymeric microparticles, alginate and PLGA are most frequently used because the preparation process is gentle and does not affect the activity of the encapsulated bioactive factors [163]. Encapsulated rhBMP-2 in alginate

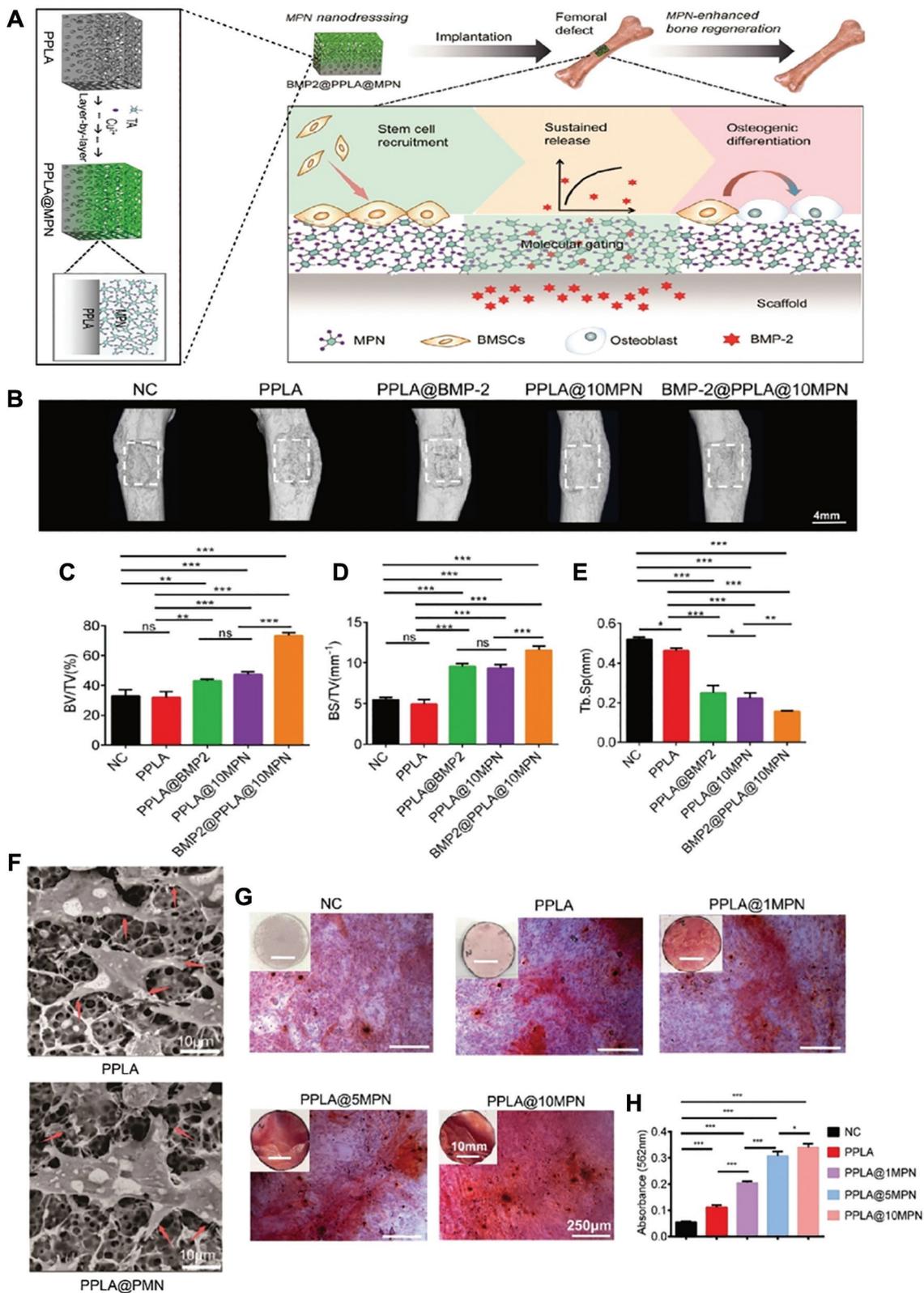
and PLGA microparticles were delivered continuously for 28 d and showed a good ability to promote repair in a rat skull defect model [164]. In one study rhBMP-2 delivered by surface-functionalized strontium alginate microparticles retained biological activity and stimulated higher alkaline phosphatase activity *in vitro*. rhBMP-2 release was sustained for approximately 3 weeks and successfully induced bone formation and biomechanical firm (a 5.3-fold increase in bone volume fraction and a 2.6-fold increase in bending stiffness [flexion/extension] compared to control) *in vivo* [165]. In addition, alginate microspheres can autonomously induce calcification *in vivo*. Crosslinked calcium alginate microspheres were implanted into nude mice and mineralized into calcium phosphate (CaP) *in vivo* through the interaction between calcium ions and physiologic phosphate ions, which was highly consistent with hydroxyapatite found in bone [166]. This method will have great potential in the application of subchondral bone regeneration.

Bone and/or cartilage repair involves a series of cascades of GFs and chemokines. Therefore, the development of particle delivery systems that combine multiple signaling molecules has become a new research direction. A dual GF delivery system consisting of PLGA and alginate was designed to differentiate MSCs into specific lineages. The microparticle delivery system can suppress the initial burst of biomolecules, resulting in the sustained release of loaded biomolecules together for up to 4 weeks. Moreover, the gene expression of osteogenic markers was significantly upregulated [167]. SOX9-loaded and TGF- $\beta$ 3-coated PLGA microspheres effectively delivered bioactive molecules to hMSCs, which resulted in upregulation of hMSC-specific marker genes and enhanced chondrogenic differentiation of hMSCs [168]. The encapsulation of IGF-I and TGF- $\beta$ 1 in PLGA microspheres clearly reduced the initial burst of both GFs and the sustained release of both GFs in biologically active forms for up to 70 d. SOX9-loaded and TGF- $\beta$ 3-coated PLGA microspheres have great potential in the application of cartilage tissue regeneration [169].

## Inorganic polymer delivery system

Studies have shown that promoting simultaneous repair of cartilage and subchondral bone is the best strategy for OC tissue regeneration. Subchondral bone is rich in blood vessels, which provide nutrients for articular cartilage and has the mechanical properties necessary for supporting the joint. Materials possessing adequate compressive strength include metallic materials, BG, and bioceramics and are the best choices for promoting subchondral bone regeneration. In addition, the ability to induce the formation of apatite is another prerequisite for the selection of materials [170].

Ceramic is the most commonly used inorganic material in bone defect repair. However, due to the lack of biological functions of ceramic materials (osteinduction and osteogenesis), various peptides, proteins, and bioactive molecules are usually modified on the surface of ceramic scaffolds to improve bone conduction [171]. The modified bioceramic scaffolds can induce the biological activity of the local microenvironment of bone tissue and provide suitable



**Figure 7** (A) Proposed mechanism of enhanced bone regeneration via MPN nanodressing of the PPLA scaffold in a femoral bone defect model. (B) Micro-CT analysis of bone regeneration in femoral defects at week 4 postoperatively. Representative 3D digital reconstructed platform images. The white color represents the repair area in the femoral bone. (C–E) Quantitative analysis of the mandibular bone by reconstruction and analysis software. (F) SEM images of BMSCs grown on PPLA and PPLA@MPN scaffolds. Red arrows indicate the extension of cellular pseudopodia. (G) Representative photographs of alizarin red staining of BMSCs in different groups at day 14 (macrographs of stained cells are inserted). (H) MPN significantly enhanced the formation of mineral deposition compared with the control PPLA groups. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ . Mean values and error bars are defined as the mean and S.D., respectively. Figure 7 was reproduced from ref [161] with permission from American Chemical Society. Copyright 2021.

mechanical strength and porosity for tissue regeneration. To enhance the osteogenic capability of hydroxyapatite/ $\beta$ -tricalcium phosphate (HAP/ $\beta$ -TCP), the ECM phosphoglycoprotein peptide (MEPE), which is known for its ability to promote osteoblast differentiation, was directly immobilized on HAP/ $\beta$ -TCP particles, then implanted into a mouse skull defect model for microCT and histologic analysis; a marked increase in bone regeneration and bone area associated with physiologic bone remodeling was observed [172]. Zhao et al. [173] designed a BMP-2-loaded calcium-deficient hydroxyapatite (CDHAP) porous scaffold and a sulfated chitosan (SCS)-coated GF delivery system. Studies have shown that SCS coating enhances the *in vitro* release properties of CDHAP/BMP-2 composites and promotes new bone formation *in vivo*. Increased bone formation was observed in both HAP/ $\beta$ -TCP-dipyridamole and HAP/ $\beta$ -TCP-BMP-2 groups when HAP/ $\beta$ -TCP was coated with BMP-2 or dipyridamole and implanted into C57B6 and A2AKO mouse models with skull bone defects with a trend toward increased remodeling [174]. In addition to peptides, the incorporation of MSCs also contributed to the overall biological function of ceramic materials. MSCs can effectively undergo osteogenic differentiation and have the ability to injure chemotaxis. Incorporating platelet-derived growth factor (PDGF) and hMSCs into HAP/TCP scaffolds improves the regenerative potential of osteocytes [175].

Another commonly used class of inorganic materials is BG. BGs have excellent bone conduction, osteogenesis, osteoinduction and mechanical strength properties. Specifically, mesoporous bioglass (MBG) has an ordered and adjustable mesoporous pore structure, a large pore volume, a specific surface area, higher drug loading, and better sustained release performance [176, 177]. Sustainable adsorption to release drug molecules for drug and GF delivery in bone regeneration has been significantly developed [178].

However, the inherent shortcomings existing in MBG materials, such as brittleness, high degradability, and surface instability, affect mechanical strength and cytocompatibility [179]. For example, combining MBG with a PLGA polymer significantly improves the biological activity, degradation rate, and controlled release performance of the material for drug delivery [180]. Wu et al. combined SF, which has good biocompatibility and tensile strength, with MBG to optimize the mechanical properties. The drug delivery and cytocompatibility of the materials were evaluated by Dex and BMSCs, respectively. The results showed that SF-MBG scaffolds could induce homogeneous and continuous Dex release and increase the adhesion, proliferation, and osteogenic differentiation of BMSCs [181].

## Controlled-release delivery system

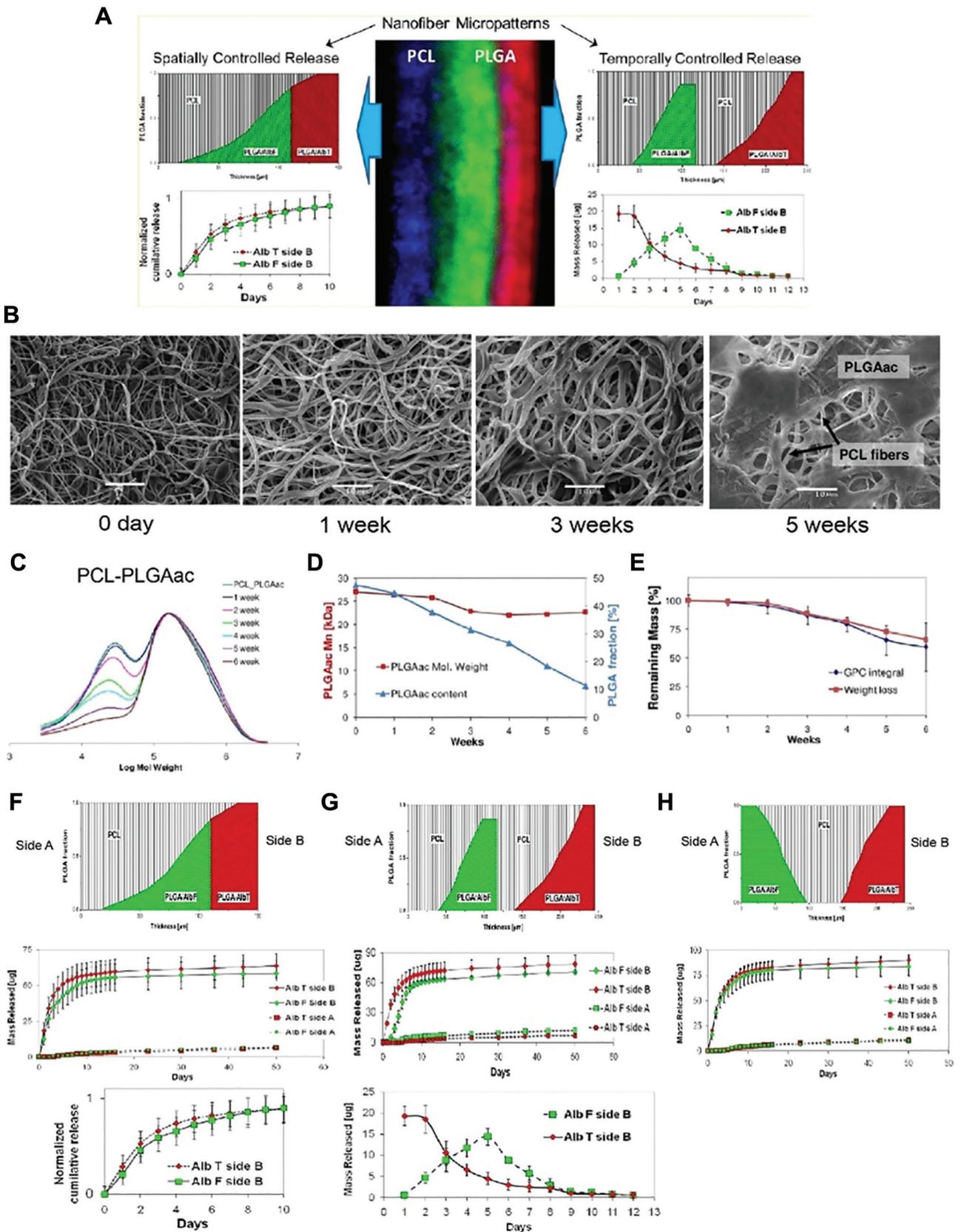
OC tissue regeneration is a cascade process regulated by a variety of cytokines and GRs. Simmons et al. designed a co-delivery system using BMP-2 and TGF- $\beta$ 3, which promoted bone formation more significantly than BMP-2 or TGF- $\beta$ 3 alone [182]. Dual delivery of VEGF and BMP-2 enhanced bone bridge and bone defect healing more than either delivery alone in a rat calvaria defect model [183, 184].

The precise regulation of drug delivery systems, including chemical modification, physical blending, and surface modification, is essential for the continuous release of bioactive molecules. Yilgor et al. [185] demonstrated that the structure of the delivery system and the mode of delivery of GFs had differential effects on the proliferation and osteogenic differentiation of MSCs. The structure of PCL and PLGA can be designed to achieve controlled and sustained delivery of one or more proteins [186]. Precise spatiotemporal control over the release kinetics of therapeutic agents (e.g., small molecules, genes, peptides, and proteins) is critical for guiding tissue regeneration in controlled delivery systems. Bonani et al. [186] engineered gradient PCL-PLGA nanofibers to achieve directional release. Confocal imaging confirmed close alignment between designed versus actual fluorescent dye distributions within the fibers, which validated gradient fidelity. During degradation, fiber hydration-induced volumetric expansion resulted in progressive diameter increase, spatially constraining molecular diffusion from the scaffold. Subsequent analysis of the PCL-PLGA delivery system time-delayed release kinetics used fluorescent-tagged small molecules and proteins as model therapeutic agents. The results demonstrated exceptional size-dependent release separation. Protein released > 88% separation efficiency at a critical scaffold thickness of 200  $\mu$ m, while small molecules exceeded 82% efficiency (Figure 8). This finding confirms precise spatiotemporal control over distinct molecular payloads. The hydroxyapatite delivery system loaded with BMP-2 and VEGF prepared using the layer-by-layer assembly (LBL) method can continuously release the two GFs. The BMP-2/VEGF composite delivery system has good osteogenesis and angiogenesis ability in the rabbit skull defect model and can effectively promote new bone formation [187].

In addition to achieving sustained and controlled delivery, the dose of the bioactive molecule loaded into the delivery system is also important. Various strategies have been devised to increase the total payload of bioactive molecules or drugs in the delivery system, allowing for sustained release over time while maintaining effectiveness. For example, delivery systems fabricated using supercritical CO<sub>2</sub> technology can enable the continuous delivery of two or more GFs at controlled doses and rates [188]. The VEGF encapsulated porous PLA delivery system prepared by Kanczler et al. can greatly improve the loading rate of VEGF without affecting its biological activity [189]. Hydrogels can also be used to construct delivery systems for controlled and sustained release of therapeutic drugs. Sustained release of BMP-2 was observed throughout the implantation period when BMP-2 was embedded in a polypropylene scaffold with PLGA microspheres surrounded by a VEGF-loaded gelatin hydrogel, which was implanted into an SD rat bone defect model [190].

## Multiphase structure delivery system

Biphasic and heterogeneous drug delivery systems have been devised to meet the apparently distinct biological and mechanical demands of cartilage and subchondral bone. These systems often utilize polymers, such as gelatin, collagen, and



**Figure 8** (A) Design illustrations and laser confocal image of the PCL-PLGA delivery system. (B) Representative SEM micrographs of the PCL-PLGA nanofibrous scaffold with an internal compositional gradient. Images were taken from the PLGA side. Changes in the nanofiber morphology after 1, 3, and 5 weeks of degradation in phosphate buffer solution at 37 °C. The unchanged PCL nanofibers underneath the PLGA layer were found after 5 weeks of degradation. (scale bar = 10 μm) GPC analyses of the as-spun PCL-PLGA after hydrolytic degradation. (C) GPC curves of PCL-PLGA. (D) Changes in the average molecular weight and remaining PLGA content determined by deconvolution of the GPC curves. (E) Overall remaining mass percentage of the scaffolds determined by the gravimetric method and measurement from GPC curves. (F) Dual-drug release from one surface of the scaffold. Illustration of the compositional pattern of nanofibers. Cumulative release curve of AlbF and AlbT. Comparison of AlbF and AlbT release during the first 10 d. (G) PCL-PLGA scaffold for the sequential release of proteins. Illustration of the compositional pattern

of nanofibers. AlbF-loaded PLGA nanofibers were confined in the middle of the scaffold, while AlbT-loaded PLGA nanofibers were close to side B. Cumulative release profiles of AlbF and AlbT to both surfaces of the scaffold. Net release profiles of AlbF and AlbT to side B during the first 12 d. (H) Dual-drug release from the opposite surfaces of the PLGA-PCL-PLGA scaffold. Illustration of the compositional pattern of nanofibers. Cumulative release curve of AlbT and AlbF. Release of green-emitting AlbF was mainly confined to side A, whereas release of red-emitting AlbT is mainly confined to side B. Figure 8 was reproduced from ref [186] with permission from American Chemical Society. Copyright 2012.

PLA, for cartilage layers, while bioceramics, such as HA and TCP, are used for subchondral bone layers within biphasic and multiphase scaffolds. Such multiphase delivery systems can create biomimetic regions resembling the layered structure and composition of natural OC tissue, facilitating the concurrent regeneration of cartilage and subchondral bone.

Jiang et al. [191] designed a multiphase delivery system comprised of an agarose gel poly-milk PLGA and 45S5 BG that enables region-specific co-culture of chondrocytes and osteoblasts to generate distinct continuous regions. The multiphase delivery system promoted chondrocyte mineralization, calcification, and subchondral bone formation. Fang et al. [192] constructed a chitosan bilayer gel delivery system with an upper layer of bFGF to induce chondrogenesis and a lower layer of uniformly distributed hydroxyapatite nanoparticles (HAPp). *In vitro* studies showed that the delivery system could promote upper chondrogenic differentiation and lower osteogenic differentiation. The system promoted both hyaline cartilage and subchondral bone formation and significantly improved osteochondral repair *in vivo*. Zheng et al. [193] constructed a bilayer delivery system containing a hydrogel upper layer and a polymeric nanofiber lower layer. The upper layer was loaded with the cartilage-inducing drug, KGN, and the lower layer was loaded with the osteogenic-inducing factor, BMP-2-derived polypeptide (P24 polypeptide). The dual-layer delivery system loaded with KGN P24 peptide can induce MSC differentiation into chondrocytes and osteoblasts, respectively. This system significantly promoted the regeneration of OC tissue in rabbit knee joints *in vivo*. A three-phase GelMA hydrogel delivery system has been developed for OC defect repair. The upper layer of the system is covalently bound to CS to promote the chondrogenic differentiation of BMSCs. The incorporation of hydroxyapatite into the middle and lower layers effectively enhanced angiogenesis and osteogenic induction. Hydroxyapatite significantly promoted cartilage and bone repair in a rabbit OC defect model [194]. Chen et al. [195] constructed a two-layer gene delivery system consisting of a chitosan-gelatin upper layer loaded with a TGF- $\beta$ 1 plasmid and a hydroxyapatite/chitosan-gelatin lower layer loaded with BMP-2 plasmid. This gene delivery system can induce MSCs to differentiate into chondrocytes and osteoblasts *in vitro*, respectively. The gene delivery system also promoted regeneration of articular cartilage and subchondral bone in a rabbit knee cartilage defect model.

## Analysis of the efficacy and limitations of these delivery systems

Hydrogel delivery systems typically utilize ECM-mimetic biomaterials that offer inherent biocompatibility, tunable

mechanical properties, and straightforward fabrication. Both natural polymers (e.g., collagen, HA, and chitosan) and synthetic polymers (e.g., PEG, PLA, and PVA) can be engineered into functional hydrogel platforms for controlled therapeutic delivery [196]. Hydrogels enable versatile loading of therapeutic agents, including small molecules, proteins, and DNA through direct integration into polymer matrices during fabrication. These systems exhibit exceptional histocompatibility and biosafety, while simultaneously preserving bioactivity and shielding payloads from enzymatic degradation. However, the inherently low mechanical strength (typically, 0.1–1 MPa tensile strength) critically limits load-bearing applications where cortical bone (> 100 MPa) or metal implants (> 500 MPa) are required. This mechanical deficiency limits utility in orthopedic procedures demanding structural integrity [197]. To overcome mechanical limitations, contemporary research focuses on functionalizing hydrogel scaffolds with compressive reinforcement, which endows rigid load-bearing capability. These engineered systems provide stable mechanical support while establishing direct osteointegration with subchondral bone, accelerating OC regeneration through biomimetic stress transfer. However, long-term toxicity of biomaterials remains critical. Degradation byproducts may exert chronic cytotoxicity or disrupt paracrine signaling. Future studies must address host-material fusion dynamics in cartilage interfaces and off-target effects on regenerative pathways, particularly for synthetic polymer hydrogels.

Metal-based delivery systems synergize high mechanical strength (0.1–1 GPa yield strength) with biocompatibility, enabling long-term use in load-bearing bone regeneration. However, the excessive Young's modulus (110–200 GPa vs. cortical bone 15–30 GPa) induces stress-shielding effects, triggering bone resorption through Wolff's law. While controlled porosity reduces stiffness toward trabecular bone levels, intrinsic limitations persist, such as non-degradability, fatigue-induced ion leakage, biofilm susceptibility, and fibrous encapsulation, which compromises osseointegration.

Nanocarriers offer tunable physicochemical properties, including precisely controlled size (10–500 nm), programmable shapes (spheres, rods, and cubes), tailored composition, adjustable surface charge ( $\pm$  5–50 mV), and high surface-to-volume ratios, enabling optimized biodistribution, cellular uptake, and therapeutic payload delivery [198]. Nanoparticles (NPs) significantly enhance therapeutic retention in cartilage while potentiating drug efficacy. These delivery systems mitigate enzymatic degradation and reduce intra-articular efflux through size-exclusion effects. Despite advantages, such as high drug-loading capacity and engineerable surface-to-volume ratios, NPs face colloidal instability risks. Critical barriers to clinical translation include undefined *in vivo* metabolic pathways and potential off-target cytotoxicity, warranting rigorous investigation of biodistribution, immune responses, and

long-term articular tissue effects. Microparticles (MPs) exhibit depot-type release kinetics analogous to NPs, leveraging the larger size for extended joint residence times. This effect enables MPs to function as combination drug banks for sustained multi-agent delivery. While MPs demonstrate reduced synovial clearance and narrower release gaps, the micrometer-scale dimensions restrict penetration beyond superficial cartilage layers, limiting therapeutic access to deep OC regions.

## Future research direction

Although new progress has been made in the delivery of bioactive molecules and drugs in recent years, the delivery of microRNAs (miRNAs) in OC tissue regeneration has rarely been studied. miRNAs are pivotal in modulating gene expression; tissue repair and regeneration are also regulated by miRNAs [199]. miRNAs have been shown to promote tissue regeneration. For example, Let-7 miRNAs can regulate peripheral nerve regeneration [200]. The multifunctional miRNA, miR-155, is involved in multiple neural repair processes in different CNS pathologic processes [201]. miR-19a/19b can enhance cardiomyocyte proliferation and stimulate cardiac regeneration after myocardial infarction injury [202]. miR-223-3p aids in skeletal muscle regeneration by regulating the equilibrium between pro- and anti-inflammatory factors within the skeletal muscle microenvironment [203]. miRNA expression were associated with protein turnover in articular cartilage tissue regeneration [204] and osteogenic and chondrogenic differentiation of MSCs [205]. These findings suggest that miRNA is a new research strategy and development direction for OC repair.

Immunomodulatory strategies are another avenue to explore tissue regeneration. The immune response to tissue injury plays a crucial role in both the pace and success of the healing process. Consequently, the manipulation of immune cells using biomaterials and drug delivery systems is emerging as an appealing strategy in the field of regenerative medicine [206]. For example, proinflammatory cytokines are detrimental to chondrogenesis and repair in cartilage tissue [207]. Some proinflammatory cytokines, such as TNF- $\alpha$ , IL-1, and IL-17, are major triggers of osteoclast activation in bone tissue and overexpression can lead to bone resorption. Osteoclast activation inhibitors, such as IL-12, IL-18, IL-33, and interferon gamma, inhibit bone resorption. Therefore, the composition of inflammatory cytokines has a decisive role in determining if bone resorption is triggered [208, 209]. This finding suggests that strategies using the regulation of inflammatory cytokines may be a novel approach to promote OC tissue regeneration. Despite a limited understanding of the underlying mechanisms of immunomodulation during tissue regeneration, the emerging field of biomaterial based immunomodulation strategies provides a new perspective for improving tissue regeneration and successfully restoring tissue function, which will promote the development of future tissue regeneration strategies [210, 211].

## Perspective summary

Despite significant progress in regenerative medicine, sustained OC repair continues to pose a major scientific and clinical obstacle. With the development of materials science, the delivery of a variety of different bioactive molecules and drugs through composite materials to promote OC tissue regeneration is the trend of future development. However, there are still many challenges that need to be addressed for osteochondrogenic drug delivery systems. Bioactive molecules are affected by the various methods used to load bioactive molecules into the delivery system (e.g., organic solvents, high temperatures, and radiation). These effects can only be compensated by a higher drug load to achieve the desired release kinetics and therapeutic effect. The effectiveness of carrier sterilization in delivery systems has also not been comprehensively evaluated. The choice of sterilization method may affect the stability of bioactive molecules, resulting in the loss of activity of biological signaling pathways.

In addition, although biphasic and multilayer structures can provide a mechanical environment like physiology for OC regeneration, it is difficult for existing biotechnology to biologically mimic the natural microstructure and physiologic properties of cartilage and subchondral bone. The adhesion strength between the two adjacent layers is often insufficient in biphasic or multiphase delivery systems, resulting in delamination of the adjacent layers. In addition, drug delivery systems incorporating bioactive molecules are difficult to store and deliver due to the instability of bioactive molecules during commercialization. Issues, such as large-scale manufacturing, precisely controlled release of bioactive molecules and high medical costs remain to be solved.

In addition to performance optimization, security is also a drug delivery system to consider. Sharafat-Vaziri et al. [212] evaluated the safety and efficacy of a collagen/SF delivery system for repairing OC defects. Tamaddon et al. [213] developed a multilayer OC delivery system for OC tissue heterogeneity and verified the safety and efficacy of the delivery system in a sheep condylar model. *In vivo* studies showed that the delivery system significantly enhanced bone growth within 12 weeks.

Currently, only a few delivery systems are used in clinical trials of OC defects, mainly including type-I/III collagen [MACI™ (Vericel Corporation, Cambridge, MA, USA) and Novocart 3D™ (B. Braun Melsungen AG, Melsungen, Hesse, Germany)], HA (Hyalograft® C, Fidia Advanced Biopolymers, Abano Terme, Veneto, Italy), PGA, PLA, and polydioxanone (BioSeed C™, BioTissue Technologies GmbH, Freiburg, Baden-Württemberg, Germany). Recently, hydrogel drug delivery systems have received much attention in the treatment of OC defects. Among the hydrogel drug delivery systems, type-I collagen hydrogels (Koken Atelocollagen Implant KOKEN CO., LTD, Tokyo, Japan), HA hydrogels (CARTISTEM™, Medipost Co., Ltd, Gangnam-gu, Seoul, South Korea), albumin and HA hydrogels (Novocart Inject™, TETEC AG, Tübingen, Baden-Württemberg, Germany), fibrin (Chondron™, Sewon Cellontech Co., Ltd, Mapo-gu, Seoul, South Korea), and agarose and alginate hydrogels (Cartipatch™, TBF Tissue

Engineering, Mende, Lozère, Occitanie, France) have wide application prospects. However, the overall clinical outcome is not satisfactory and more high-level studies and longer follow-up are needed.

In summary, an optimal drug delivery system needs to enhance multiple characteristics, such as size, encapsulation efficiency, deformability, thermal responsiveness, biocompatibility, biodegradability, and cost-effectiveness. How to select the appropriate biomolecules, delivery systems, and processing technologies to construct the optimal therapeutic system and achieve the ideal tissue regeneration effect requires complex design and engineering.

An ideal drug delivery system maintains its physical properties in the OC tissue and supports chondroprogenitor cell proliferation by controlling the release of bioactive molecules to promote chondroprogenitor cell differentiation. In addition, an ideal delivery system should mimic the biomechanics, porosity, and hierarchical microstructure of the OC interface with degradation timing synchronized with the rate of new tissue formation.

## Data availability statement

Not applicable.

## Authors' contributions

Conceptualization, J.S. and B.L.; writing—original draft preparation, J.S., J.Z., and L.L.; writing-review and editing, Y.Z., C.L., and B.L.; visualization, J.S., J.Z., L.L., W.K., P.Z., G.S., H.Z., and W.S.; supervision, C.L. and B.L.; project administration, C.L. and B.L.; and funding acquisition, J.R.,

C.L., and B.L. All authors have read and agreed to the published version of the manuscript.

## Ethical approval and consent to participate

No human or animal studies were performed by the authors. Therefore, ethical approval and informed consent were not required.

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## Conflict of interest

Dr. Chaorong Liu is the Co-Editor-in-Chief of *BIO Integration*. He was not involved in the peer review or handling of the manuscript. The other authors have no other competing interests to disclose.

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