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## 1. Introduction

The knee joint is the largest weight-bearing joint in the human body and the most prone to articular cartilage damage due to factors such as excessive frequency of use, complex structure, and strong weight-bearing. Once the articular cartilage is damaged, it is difficult to repair and susceptible to degenerative changes in the joints.<sup>1,2</sup> The surfaces of connecting bones in a joint are covered with a layer of articular cartilage, which is often worn away by the shear forces generated by joint movement. Articular cartilage has no blood vessels or lymphoid tissue; its blood supply comes mainly from the synovial fluid and subchondral bone in the joint cavity. As a result, it is difficult to completely regenerate articular cartilage, and if it is damaged, it can lead to permanent damage.<sup>3,4</sup> Knee cartilage injury is a common orthopedic disease. Its main clinical manifestations are joint pain and activity dysfunction. According to the International Cartilage Repair Association for grading articular cartilage injury standards, articular cartilage defect exists when the diameter of the defect is  $>3$  cm. This type of defect cannot be completely self-repaired and also further damages the surrounding cartilage tissues and the surrounding wall of the bone, causing joint inflammation or articular cartilage collapse, eventually evolving into osteoarthritis.<sup>5</sup> Relevant studies have found that the incidence of knee cartilage damage in adults with non-symptomatic knee osteoarthritis (mean age

= 62.3 years) is 69%, and the incidence of knee cartilage defects in patients with symptomatic knee osteoarthritis (mean age = 66 years) is  $>80\%$ .<sup>6,7</sup> Currently, clinical surgery is used as the main treatment for knee cartilage defects.<sup>8</sup>

Tissue engineering is a multidisciplinary field involving cells, biomaterials, cell–material interactions, and their characterization through different surface technologies, which can be combined to obtain better treatments that facilitate the regeneration of new tissues.<sup>9</sup> Hydrogels, as a material, are tunable, degradable, biocompatible, and modifiable, offering numerous advantages in tissue engineering and cell delivery applications.<sup>10,11</sup> However, due to their inherently poor mechanical properties, they are not suitable for applications requiring high strength.<sup>12</sup> Additionally, the rapid biodegradation of hydrogels significantly limits their broader application in the field of tissue engineering.<sup>13</sup> Therefore, improving them through different methods can further enhance application in tissue engineering.<sup>14</sup> Hydrogel composite systems are an approach for creating materials that are superior to any single hydrogel, obtained by combining the functions and properties of different hydrogel materials. In past decades, various composite designs have been proposed to enhance the properties of hydrogels, which include particles, anisotropic fillers, and fiber-hydrogel composite systems.<sup>15,16</sup> These reinforcements are immobilized in a hydrogel matrix through physical or chemical interactions to enhance the hydrogel properties. In hydrogel composites, multiple polymer networks interpenetrate with each other, resulting in mechanical anchoring behavior, which can significantly affect the rheology, degradation rate, permeability, and mechanical properties of the hydrogel.<sup>17</sup>

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Owing to the rapid progress and extensive utilization of hydrogel composite scaffolds in biomedical arenas, they hold significant potential in cartilage tissue engineering. In this comprehensive review, we commence by methodically outlining the structure of the knee joint. Thereafter, we dissect the principles and recent research advancements of frequently employed surgical techniques for treating knee cartilage defects. These encompass microfracture, chondroplasty, osteochondral autotransplantation, autologous matrix-induced chondrogenesis, and osteochondral allograft. Subsequently, we engage in an in-depth discussion regarding different varieties of hydrogel composite scaffolds, namely, natural hydrogel composite scaffolds, nanoparticle composite hydrogel scaffolds, nanotube composite hydrogel scaffolds, and microsphere composite hydrogel scaffolds. We explore their physical attributes, functional characteristics, and associated preparation procedures. Eventually, we appraise the extensive employment of hydrogel composite scaffolds in the treatment of knee cartilage defects and conclude with a summary and projection of future trends and directions.

## 2. Cartilage structure of the knee joint

Knee joint is the interconnection of the thigh and lower leg and consists of the patella, distal femur and proximal tibia. On the articular surface of all three bones, there is a layer of smooth, transparent cartilage.<sup>18</sup> The cartilage of knee joint is mainly composed of surface cartilage, intermediate cartilage, deep cartilage and subchondral bone.<sup>19</sup> Surface cartilage is located in the outermost layer, which is the site of direct contact between joints and has a smooth surface. Intermediate and deep cartilages are located below the surface cartilage and composed mainly of collagen fibers and proteoglycans, which provide elasticity and cushioning. The subchondral bone is located at the base of the cartilage and provides support and nutrition to it.<sup>20,21</sup>

Histologically, articular hyaline cartilage, including patellar cartilage, femoral condylar cartilage, and tibial plateau cartilage, is a semi-solid, supportive connective tissue mainly

composed of chondrocytes and cartilage matrix. The main chemical components are cartilage mucin, water, and type II collagen fibers in the highest content, which are cross-linked to form a lattice scaffold wherein water and mucins are embedded. This structure is extremely important for the mechanical stability of cartilage required for weight bearing.<sup>22,23</sup> Chondrocytes, collagen fibers, and ECM together form the different regions of articular cartilage, dividing it into a four-layered structure of superficial, intermediate, deep, and calcified layers.<sup>24,25</sup> Most of the collagen fibers in the superficial layer are parallel to the articular surface, and this layer has the lowest content of proteoglycan and chondrocytes but is rich in water. The intermediate layer is the transition layer; the collagen fibers are distributed in a staggered fashion. The deep layer is the radiolucent layer; the collagen fibers are roughly distributed perpendicularly to the articular surface in a radial pattern, and this layer is rich in proteoglycan, with the lowest content of water and the highest content of collagen fibers, which is the largest and thickest and the most important component of articular cartilage. The deepest layer is the calcareous cortex, where the collagen fibers are arranged in a reticular pattern and extend toward the cortical bone surface (Fig. 1).<sup>26,27</sup>

## 3. Knee cartilage defects and repair

Knee cartilage defects, such as the medial femoral condyle, lateral femoral condyle, or patellofemoral compartment, may be caused by mechanical factors (e.g., trauma or repetitive microtrauma) or biological factors (e.g., exfoliative osteochondritis dissecans or osteonecrosis).<sup>28</sup> The pain, swelling, and dysfunction caused by knee cartilage defects are more severe compared to anterior cruciate ligament injuries or even knee arthroplasty.<sup>29</sup> Defects in the weight-bearing portion of the femoral condyle result in increased cartilage wear at the margins of the lesion and reduced contact area, edge loading, and increased cartilage stress in the adjacent region resulting from full-length cartilage defects are considered to predispose this tissue to degenerative changes.<sup>30,31</sup> These defects usually result in the production of type I collagen in the fibrocartilage,<sup>32</sup> which is

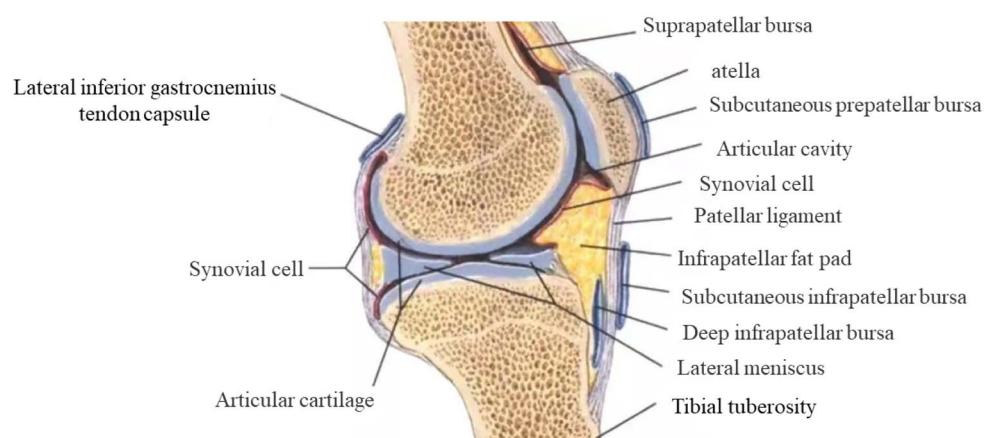


Fig. 1 Anatomy of knee cartilage.



characterized by poor elasticity, stiffness, and wear properties, making it susceptible to arthritis.

The classification according to the Anderson Orthopedic Research Institution (AORI) criteria divides the types of bone defects into three categories: type I bone defects, with only mild loss of osteophytes, no osteolysis or prosthetic subsidence, and an intact metaphysis; type II bone defects, which can also be classified as unilateral or bilateral, with metaphyseal femoral condylar or tibial plateau bone defects, with a small amount of osteolytic defect at the distal end of the femoral epicondyle, upward displacement of the joint line or sinking of the femoral end prosthesis, shortening of the metaphysis, and sinking of the tibial end prosthesis below the fibular head. Type III bone defects affect the attachment of structures such as the surrounding ligaments, with the defect or prosthesis sinking to the level of the cavernous tuberosity and reaching or exceeding the level of the femoral condyles, resulting in a large segmental defect in the metaphysis, which is often associated with lateral collateral ligament injuries.<sup>33,34</sup> The goal of surgical treatment of these defects is to reconstruct the hyaline cartilage on the articular surface to achieve a properly aligned and stabilized joint, thereby relieving symptoms and reducing the risk of further progressive arthritis. The surgical approaches that exist in clinical practice are microfracture, chondroplasty, osteochondral autografting, autologous matrix-induced chondrogenesis, and osteochondral allografts (Table 1).

### 3.1 Microfracture

Microfracture technique is a commonly used treatment for intra-articular cartilage lesions in the knee joint involving the formation of multiple perforations or microfractures in the subchondral bone plate, which penetrates the cartilage defect, causing bleeding and subsequent fibrin clot formation, defect filling and covering the exposed bone surface, thus allowing bone marrow MSCs to migrate into the clot from the bone

marrow underneath to the articular surface to form a bone and fibrous cartilage mixture and promote the formation of fibrocartilage repair tissue.<sup>31</sup> The biochemical and biomechanical properties are poor compared to normal hyaline cartilage, and although early short-term improvement in symptoms has been shown, there are drawbacks related to the durability and longevity of fibrocartilage repair.<sup>35</sup> Studies have reported an 80% survival rate at an average of 9.4 years after microfracture of focal cartilage humeral head defects with a minimum of 5 years of follow-up.<sup>35</sup> Evaluating results 10–14 years after the treatment of microfractures of focal cartilage defects in the knee showed a poorer prognosis.<sup>36</sup>

To overcome the mechanical limitations of microfracture methods, nanofracture techniques have been gradually developed, which have the advantage of a denser distribution of microfractures, lesser damage to the subchondral layer at the defect site, and no thermal damage to osteocytes during the procedure.<sup>37</sup> At the same time, deeper subchondral penetration leads to more efficient filling of the defect with extravasated blood and a higher proportion of fibrocartilage occurs at the site of injury.<sup>38</sup> In subchondral bone remodeling after the treatment of total cartilage defects with nanofractures in an animal knee model, it was found that nanofractures resulted in deeper subchondral bone penetration, less trabecular fragmentation and compaction, and better restoration of normal subchondral bone structure at six months.<sup>39</sup>

In conclusion, microfracture is the cheapest and simplest treatment and yields significant clinical results in the short to medium term treatment of smaller cartilage defects.<sup>35</sup> If microfracture fails, other cartilage repair options can still be used to treat cartilage lesions.<sup>8</sup>

### 3.2 Chondroplasty

Chondroplasty can be performed by repairing knees having cartilage defects with healthy cartilage. This method involves

Table 1 Clinical surgical approaches to repair cartilage defects in knee joints

Types	Advantages	Disadvantages
Microfractures	Easy to operate; can effectively reduce acute or chronic knee cartilage defect pain symptoms	Poor abrasion resistance; may increase subchondral cyst formation and bone fragility; affects re-treatment
Chondroplasty	Preserves the part of damaged cartilage, which can reduce the damage to normal cartilage; it can promote the proliferation and repair of chondrocytes, helping to restore cartilage functions	Limited by cartilage repair capacity, results may be limited in cases of large cartilage defects; higher surgical complexity; longer postoperative recovery time
Osteochondral autograft transplant	Better graft survival; effective in restoring texture, thickness and elasticity of knee cartilage	Limited area, scope, and number of extractions; prone to osteomalacia in the cartilage donor area
Autologous matrix-induced chondrogenesis	Less risk of immune reaction and rejection caused by allografts or synthetic materials; higher success rate of chondrogenesis; based on the body's natural process of cartilage regeneration, which promotes chondrocyte proliferation and differentiation by providing structural support and cell growth factors	Longer recovery period; patients may experience short-term pain and discomfort
Osteochondral allografts	Adequate source of grafts; good biomechanics	May cause immune rejection and complications



debridement by machinery to remove the free edges to stabilize the lesion and potentially stimulate healing, but it can lead to persistent fissures and uneven surface formation.<sup>40,41</sup> Radiofrequency thermal ablation uses a plasma energy field for debridement, which can produce a more homogeneous articular surface, thus providing a better sliding surface.<sup>42,43</sup> One study reviewed patients who underwent BMAC chondroplasty and examined the efficacy of delaying further intervention. Results showed that only 5 of 23 procedures (21.7%) required postoperative interventions during the two-year follow-up period, and only one request for a total knee arthroplasty was made, which has not yet been completed, demonstrating that BMAC chondroplasty may be an effective method for delaying total knee arthroplasty.<sup>44</sup> It has been suggested that chondroplasty with plasma layers used in conjunction with appropriate settings and techniques could be a safe surgical method for the treatment of intra-articular ICRS grade 2 and 3 lesions in the knee, with the primary goal of slowing the progression of cartilage lesions and improving patient prognosis, and that plasma layers may be a reasonable option for the treatment of patients with grade 2 and 3 lesions.<sup>40</sup> Despite the high prevalence of chondroplasty, there are limited short- and long-term outcome data. To date, the procedure has only been described as a primary treatment or concomitant surgical intervention for osteoarthritis (OA), most commonly, subtotal meniscectomy.<sup>45</sup>

### 3.3 Osteochondral autograft transplant (OAT)

Osteochondral autograft transplant (OAT) is an attractive treatment for small-to medium-sized cartilage and osteochondral lesions of the knee. Cartilage and subchondral bone are typically obtained from the autologous non-weight-bearing articular surface to repair cartilage defects, and a healthy peg post containing articular cartilage, cartilage tidemarks, and subchondral bone is transplanted into an area of the injury with matching size.<sup>46</sup> The advantages of this technique include the use of articular hyaline cartilage rather than fibrocartilage to repair the defect and maintenance of the height and shape of the joint.<sup>47</sup> The bony portion of the graft usually heals completely with the surrounding bone, whereas the cartilage surface, although viable, may not heal completely with the surrounding bone.<sup>48</sup> Autografts can provide excellent skeletal support for overlying hyaline cartilage, and the use of single or multiple grafts to achieve repair can cover recipient defects, with the space between osteochondral plugs filled with fibrocartilage, while the donor site may be empty and show fibrous tissue filling.<sup>49</sup> An evaluation showed that the time from symptom onset to surgery, number and size of lesions, location and quality of the surrounding cartilage, and concomitant meniscal damage influence postoperative outcomes.<sup>50</sup> In a sample study of 1139 patients (532 for open OAT and 607 for arthroscopic OAT), the size of the defect in open OAT was three times larger than that in arthroscopic OAT ( $2.96 \pm 0.76$  vs.  $0.97 \pm 0.48 \text{ cm}^2$ ). Regarding defect site, the medial femoral condyle (MFC) was the most common (75.4%), followed by lateral femoral condyle (LFC, 12.1%) and patella (6.7%). Clinical outcomes were generally favorable for open and arthroscopic

OAT; however, open OAT allowed the treatment of lesions approximately three times the size of arthroscopic OAT.<sup>46</sup> OAT demonstrates a significantly lower rate of symptomatic recurrence at 10 years postoperatively compared to the microfracture technique.<sup>51</sup>

### 3.4 Autologous matrix-induced chondrogenesis (AMIC)

Since the fibrin network of blood clots derived from microfractures containing MSCs may not be stable enough to withstand the biomechanics of the knee joint, this predisposes it to premature breakdown under normal knee loading.<sup>52</sup> Microfracture techniques are usually recommended for small cartilage defects, but autologous matrix-induced chondrogenesis (AMIC) technique offers an effective solution for larger defects.<sup>53</sup> AMIC is a matrix-assisted bone marrow stimulation technique that combines natural collagen I/III with microfracture for the treatment and repair of osteochondral defects.<sup>54</sup> The matrix stabilizes and protects the bone marrow clots generated by MSC-producing microfractures with a superficial dense layer that acts as a smooth barrier surface to prevent MSCs from spreading into the joint and a deep porous layer that promotes cellular uptake and adhesion.<sup>55</sup> The procedure employs cell-free microstructured scaffolds to fill the defect after debridement and stimulation, and adherence of the covering with sutures or fibrin glue to capture bone marrow cells and stem cells, providing a scaffolding network on which cartilage can grow, which requires neither cartilage harvesting nor *in vitro* cell expansion.<sup>56</sup> Analysis with at least two years of follow-up showed that the subjective IKDC score was better in the AMIC group than in the microfracture group. In addition, MOCART scores from MRI scans and acceptable defect fill rate results showed AMIC to be superior to MFx.<sup>56</sup> After enrolling 101 patients aged 12 to 60 years with a mean follow-up of 30 months, AMIC was proved clinically and radiologically effective after at least 2 years, with improvements in all SF-36, KOOS and IKDC domains and a higher mean MOCART 2.0 score.<sup>57</sup> Randomized controlled trials have shown that AMIC prevents the regression of common outcomes in microfractures, with 5 year outcomes significantly better than those for microfractures.<sup>58</sup>

The AMIC technique provides mechanical stability to knee cartilage defects and allows stromal cells to differentiate into chondrocytes, making it a relatively simple and attractive treatment. However, further research and refinement of this technique are needed for intermediate to late clinical outcomes.

### 3.5 Osteochondral allografts (OCA)

Osteochondral allografts (OCA) complement other revision procedures that present poor quality articular cartilage repair tissue or poor quality subchondral bone.<sup>59</sup> OCA utilize fresh, fresh-frozen, or cryopreserved allograft specimens to fill defects, and due to the presence of intact hyaline cartilage, fresh OCA do not require a blood group match or a human leukocyte antigen match. At the same time, they preclude the need to obtain material from non-weight-bearing areas and can cover virtually all sizes of defects. They also provide the possibility of restoring complex defect surfaces with no lesions without causing donor



site morbidity.<sup>60,61</sup> OCA are commonly used not only for femoral condylar defects but also for tibial plateau and patellofemoral defects.<sup>62</sup> Long-term (>10 years) graft survival has been shown to be between 70% and 91%, with excellent therapeutic results in medium to large cartilage lesions.<sup>63</sup> However, the short shelf life and high cost of fresh samples may limit their availability, cryoprocessing may result in up to 95% chondrocyte loss, the use of OCA may lead to recipient rejection, and smaller or thinner grafts may fail to heal and consolidate.

## 4. Hydrogel composite scaffolds

Hydrogel composite systems are one of the most suitable strategies for combining and assembling various hydrogel functions and properties. They are fabricated as hydrogel composite scaffolds with improved mechanical properties and biological functions that cannot be achieved by any single hydrogel and modified into different types of composite scaffolds to address limitations according to different tissue engineering needs.

### 4.1 Natural-synthetic hydrogel composite scaffolds

Natural-synthetic composite hydrogels are prepared by block copolymerization or physical interactions between natural and synthetic polymers and have good biocompatibility and mechanical properties (Table 2). Such scaffolds provide the support structure required for cell growth and tissue repair and are capable of gradual degradation to promote new tissue generation. The performance of scaffolds can be modulated by adjusting the composition and structure of the material. A wide variety of natural and synthetic hydrogels made from gelatin, collagen, alginate, hyaluronic acid and polyethylene glycol have been reported.<sup>96</sup> For example, filipin protein assembled with tannic acid (TA), polyvinyl alcohol (PVA) and chitosan combined by physical cross-linking in a KOH/urea solubilization system to prepare new dual-network hydrogels show improved hydrogel properties and enhanced bone defect regeneration.<sup>97,98</sup> Lee *et al.*'s crosslinked gelatin, prepared by enzyme-crosslinked hydroxyphenylpropionic acid (GHPA) coupling, significantly induced MSC endothelial cell growth and differentiation and was also reported to reduce the activation of host macrophages.<sup>99</sup> Gelatin methacrylate-based (GelMA) hydrogels have been widely used to construct bone repair material systems due to their tunable mechanical properties, excellent photo-crosslinking ability, good biocompatibility, and ability to promote bone differentiation and vascularization.<sup>100</sup> However, due to the lack of osteogenic activity, GelMA hydrogels were combined with other types of materials with osteogenic activity, such as bioceramics, bioglasses, biomimetic scaffolds, inorganic ions, biomimetic periosteums, growth factors, and two-dimensional (2D) nanomaterials to improve the osteogenic capacity of current composites. Lu *et al.* designed a new GelMA-HAMA/nHAP composite hydrogel as a drug delivery system where EXO was encapsulated in the hydrogel and released slowly for better osteogenesis. The results of *in vitro* and *in vivo* studies showed that this controlled and biocompatible EXOs/

GelMA-HAMA/nHAP composite hydrogel could effectively promote bone regeneration by coupling osteogenesis and angiogenesis.<sup>101</sup> However, further studies and optimization are needed to improve the performance and adaptability of the scaffolds to address issues such as scaffold stability and immune response.

### 4.2 Nanoparticle composite hydrogel scaffolds

Nanoparticles have been widely used in local drug delivery systems not only for their excellent bioactivity, promotion of bone marrow stem cell adhesion and expression of bone-related genes but also their enhanced pharmacological effects.<sup>102,103</sup> Conventional hydrogels have limited mechanical strength and are prone to fracture, further limiting their functionality, while combination with nanoparticles expands the range of hydrogel applications. As a result, nanoparticle hydrogel hybrids typically exhibit better bioactive reservoir capacity and provide appropriate controlled release rates of drugs and factors. In addition, the incorporation of nanoparticulate elements into the hydrogel network expands the range of achievable mechanical properties while improving localized nanoparticle retention.<sup>104,105</sup> Hydrogels are often combined with nanoparticles such as organic polymer nanoparticles, inorganic nanoparticles, and metal/metal oxide nanoparticles for superior performance.<sup>106</sup> The preparation of nanoparticle-composite hydrogel scaffolds usually involves mixing nanoparticles with a hydrogel prepolymer, followed by a gelation reaction to form a gel structure. Nanoparticles can increase the mechanical strength and stability of the scaffolds and also modulate the performance of the scaffolds by adjusting the type, concentration and distribution of nanoparticles. In addition, nanoparticles can be surface-modified to achieve functionalization of the scaffolds, leading to properties such as drug slow release and bio-recognition. Common organic polymer nanoparticles include polyvinyl alcohol, poly(methyl methacrylate), polylactic acid, polyacrylic acid, and polystyrene, and inorganic nanoparticles include hydroxyapatite, silica, silicate, and calcium phosphate. Metal/metal oxide nanoparticles include gold, silver, and iron oxide, which are widely used due to their corrosion and oxidation resistance, high specific surface area, surface chemistry and functionalization.<sup>107</sup> The incorporation of magnetic metal nanoparticles leads to higher viability and osteogenic differentiation of MSCs.<sup>108</sup> For example, the doping of hydroxyapatite in filipin hydrogels promoted the osteogenic differentiation of human MSCs.<sup>109</sup> In another study, multifunctional nanocomposite hydrogels were constructed by covalently binding silver ( $Ag^+$ ) core-embedded mesoporous silica nanoparticles ( $Ag@MSN$ ) to bone morphogenetic protein-2 (BMP-2), which was encapsulated into silk fibroin methacryloyl (SilMA) and photo-crosslinked to form an  $Ag@MSN$ -BMP-2/SilMA hydrogel to preserve the biological activity of BMP-2 and delay its release. These hydrogels possess synergistic osteogenic and antibacterial effects to promote bone defect repair.  $Ag@MSN$ -BMP-2/SilMA exhibited good biocompatibility *in vitro* and *in vivo* owing to its interconnected porosity and improved hydrophilicity.<sup>110</sup> The enhancement of material properties can be realized



Table 2 Types and characteristics of natural and synthetic hydrogels

Types	Material	Application	Advantages	Disadvantages	Ref.
Natural hydrogel	Gelatin	Drug delivery	High drug loading capacity; control the release rate; biocompatible; Good drug stability, which can ensure the supply of oxygen and nutrients to cells in tissue engineering; and promote the growth and differentiation of cells; it can be used to carry and release bioactive factors; adjustable physical properties; strong plasticity and manufacturability	Affected by temperature; the mechanical properties need to be improved	64–69
	Tissue engineering		Good biocompatibility; good air permeability, which can ensure the supply of oxygen and nutrients to cells in tissue engineering; and promote the growth and differentiation of cells; it can be used to carry and release bioactive factors; adjustable physical properties; strong plasticity and manufacturability	Low mechanical strength; water solubility; lack of orientation; it takes a long time to achieve tissue repair and regeneration	
Regenerative medicine			It has good biocompatibility and can reduce foreign body rejection; bioactive substances (such as growth factors and extracellular matrix components) can be added to promote cell attachment, proliferation and differentiation, which can help tissue regeneration; it has a good three-dimensional structure and can provide a microenvironment that supports and guides cell growth	Relatively low mechanical strength; rapid biodegradation; the cost is higher; poor stability in the aqueous environment	
Cell culture			Good biocompatibility; three-dimensional support structures are available; regulation performance; injectability	Limited mechanical properties; poor dynamic stability; degradation rates are inconsistent	
Hyaluronic acid		Drug delivery	Low toxicity; good biodegradability; promotes drug absorption and stabilizes the effect of the drug	Low viscosity and high dynamic viscosity; affect the flow performance of the drug; the preparation process is complex and costly	70–74
Tissue engineering			High biocompatibility; promotes tissue regeneration; it can prevent chronic inflammatory reactions	Rapid degradation rate; poor mechanical strength; susceptible to the decomposition and attack of a variety of enzymes; the activity <i>in vivo</i> is short	
Regenerative medicine			Good biocompatibility; promotes tissue regeneration and cell regeneration; biodegradable; it strengthens tissue gaps	Weak machinery; high cost	
Cell culture			It can support cell proliferation, cell differentiation and embryonic development and promote cell-to-cell communication	Lack of indicative, directed alteration of differentiation; the cost is higher	
Alginic acid		Drug delivery	High biocompatibility; good degradability; controlled-release medications; protects the drug from degradation and stabilizes the effects of the drug; having bioadhesion can increase drug adhesion and adsorption capacity	It is easy to lose drugs under acidic conditions, and it is somewhat restrictive to some drugs, which may reduce their activity	75–78
Tissue engineering			Adjusts the rheological properties of the gel; high biocompatibility; supportive cell adhesion and proliferation; promotes tissue regeneration	Rapid biodegradation; the mechanical strength is relatively low, and the stability and mechanical properties of the material need to be further improved	

Table 2 (Contd.)

Types	Material	Application	Advantages	Disadvantages	Ref.
Collagen	Regenerative medicine		Good biocompatibility; degradability; supportive cell adhesion and proliferation; may promote tissue regeneration	Poor stability	
	Cell culture		Sertoli cell attachment and proliferation; it can promote cell-to-cell communication; regulates cell differentiation and function	Due to the gelling properties and pore structure of alginic acid, it may affect the morphology and diffusion of cells	79–81
	Drug delivery		Good biocompatibility, good biodegradability, strong effect on drug stability, can reduce toxicity, and controllable release of drugs	Unstable at a certain temperature and pH; not suitable for all drugs, antigenic	
	Tissue engineering		Good biodegradability; supportive cell adhesion and proliferation; provision of structural frameworks; the composition is similar to that of the extracellular matrix	The biological activity is short and the stability is poor	
	Regenerative medicine		Good biocompatibility, biodegradable; sertoli cell attachment and proliferation; promotes tissue regeneration	Poor machinery	
	Cell culture		Sertoli cell attachment and proliferation; it can promote cell-to-cell communication; regulates cell differentiation and function	The cost is higher	
Synthetic hydrogel	Drug delivery		Good biocompatibility, biodegradability, high stability, releasable drugs, increases the stability and solubility of drugs; the way the drug is delivered can be adjusted by changing its molecular weight and chain structure	Immunogenicity is present and may elicit an immune response	82–84
	Tissue engineering		Good biocompatibility, adjustable structure and physical properties of scaffolds, supports cell adhesion and proliferation, and promotes tissue regeneration; its biological activity and cellular affinity can be enhanced by chemical modifications	Slow biodegradation, poor mechanical properties, lack of cell signaling and biological activity	
	Regenerative medicine		Biocompatible, biodegradable, supports cell attachment and proliferation, provides physical support and structural framework; its biological activity and tissue regeneration ability can be enhanced by chemical modification	It can lead to immune and inflammatory responses, and the biological stability and safety of the material need to be considered	
Polylactic acid	Drug delivery		Biodegradable, good stability, reduced toxicity, controllable release of drugs	Instability at a certain temperature and pH may lead to inactivation or inconsistent release of the drug	85–87
	Tissue engineering		It has high compatibility, good biodegradability, and supports cell and tissue growth	The rate of degeneration should be controlled to avoid affecting tissue regeneration	
	Regenerative medicine		Biodegradable, supports cell and tissue growth and regeneration, and promotes healing	Degradation rates and mechanical properties are inconsistent	
Polyglycolic acid	Drug delivery		High biocompatibility, good biodegradability, controllable release of drugs, and improved drug bioavailability	The molecular structure is prone to breakage, making it difficult to produce a sustainable release effect	88–90

Table 2 (Contd.)

Types	Material	Application	Advantages	Disadvantages	Ref.
		Tissue engineering	Good biocompatibility and biodegradability	Acids are produced during the degradation process in the body. The biodegradation process is slow and can cause stress on the regenerating tissue	
		Regenerative medicine	Good biocompatibility and biodegradability		
Polyethylene oxide	Drug delivery		It is biocompatible, degradable, can form stable water-soluble complexes, and can regulate the release rate and behavior of drugs	Solubility and stability are affected by factors such as pH, temperature, and concentration	67, 91 and 92
		Tissue engineering	Biocompatible, degradable, three-dimensional structure that supports and guides cell and tissue growth	Cytotoxicity	
		Regenerative medicine	Biocompatible, degradable, three-dimensional structure that supports and guides cell and tissue growth	The degradation products are toxic	
Polycaprolactone	Drug delivery		Good biocompatibility, good biodegradability, controllable drug release, adjustable melting temperature, easy processing	The rate of drug release is slower and needs to be modulated in combination with other materials or modified methods	93–95
		Tissue engineering	High biocompatibility, good biodegradability, and good mechanical properties of materials	During the biodegradation process, the mechanical stability of the material is gradually lost	
		Regenerative medicine	High biocompatibility, good biodegradability, adjustable melting temperature, can form complex three-dimensional structure; the mechanical properties and morphological stability of materials also provide good support for regenerative medicine	The rate of degradation is slower and may affect the regeneration and repair of tissues	

by combining hydrogels and NPs. At the same time, the limitations of hydrogel scaffolds, such as poor mechanical strength and lack of bioactivity, can be overcome (Fig. 2).<sup>16</sup> Nanoparticle composite hydrogel scaffolds have a wide range of applications and can be used to improve therapeutic efficacy in medicine and biology.

### 4.3 Nanotube composite hydrogel scaffolds

Nanotube composite hydrogel scaffolds are biomaterials obtained by fusing inorganic nanomaterials nanotubes with biomaterials hydrogels, and they are widely used in biomedical fields such as tissue engineering and biosensing.<sup>111</sup> Single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) are the two most widely used types of carbon nanotubes, which have tunable chemical and mechanical properties such as electrical conductivity, biocompatibility, and nanoscale size.<sup>64,112</sup> The high specific surface area and porous structure of nanotubes facilitate cell attachment, proliferation, and differentiation. Bioactivity on the surface of nanotubes can induce the differentiation of chondrocytes to

chondrocyte-like cells and promote the regeneration and repair of cartilage tissues.<sup>113</sup> Hydrogel scaffolds, with their soft structure and high degree of hydration, can mimic the softness and porous structure of human cartilage, while the addition of nanotubes can enhance the mechanical properties of the scaffolds, provide additional mechanical support, and guide the growth and distribution of cartilage tissues, which can help to form functional cartilage tissues. Meanwhile, the porous structure and high specific surface area of the nanotubes endow them with good drug adsorption and release properties. The scaffolds can carry drugs or growth factors, and by controlling the rate and mode of release, the proliferation and differentiation of chondrocytes and the repair and regeneration of cartilage tissue can be enhanced.<sup>114</sup> Studies have shown that nanotubes have good compatibility with chitosan, which not only improves the mechanical properties of the material but also its wettability, and combination with chitosan has little effect on the pore structure but increases the number of pores, thus promoting cell survival.<sup>115</sup> Kazemi-Aghdamfen *et al.* synthesized chitosan-modified halloysite nanotubes (mHNTs),

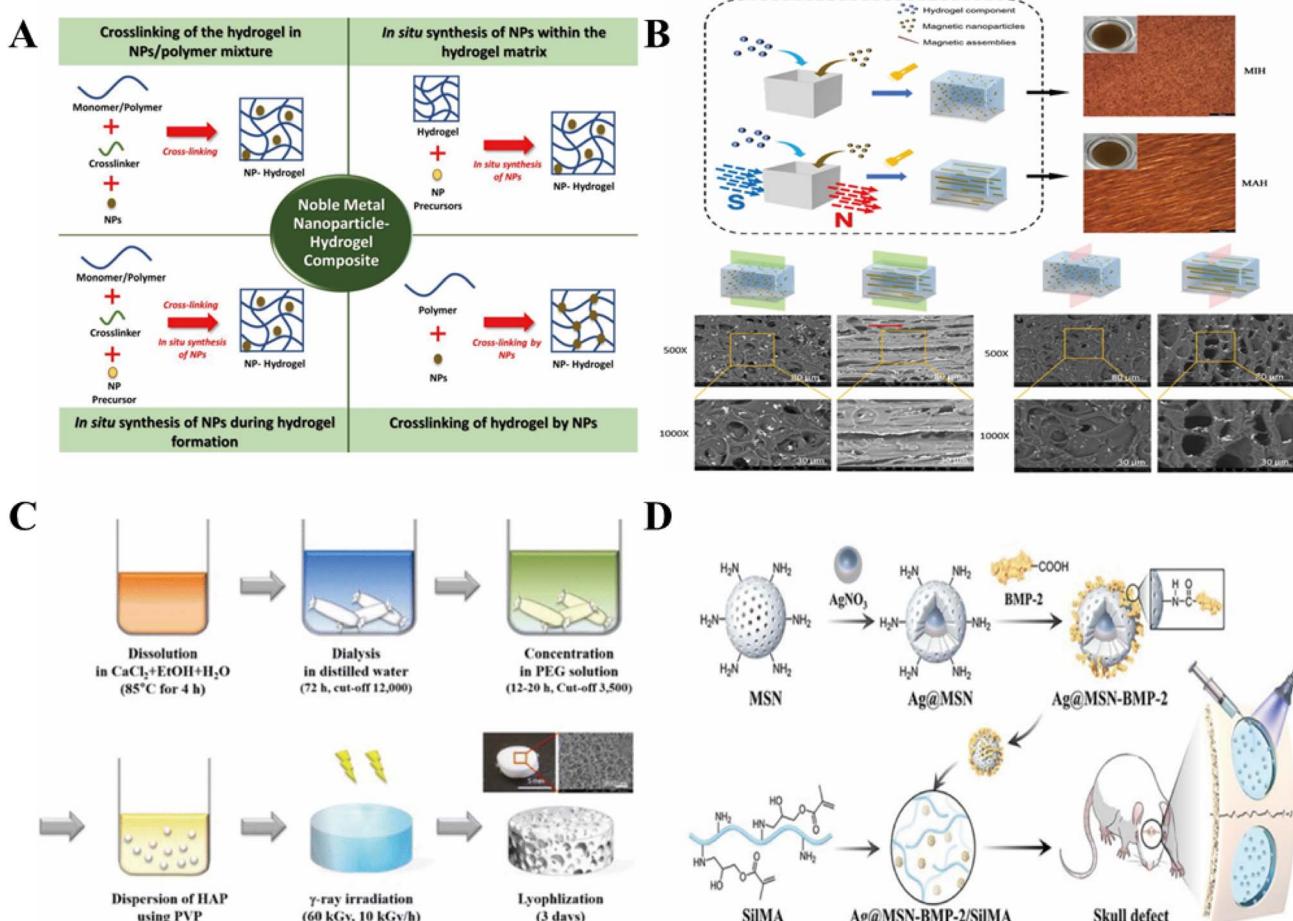


Fig. 2 Application of nanoparticle composite hydrogel scaffolds. (A) Metal nanoparticles (NPs) with hydrogel preparation. Reprinted with permission from ref. 107. Copyright 2019 MDPI, Basel, Switzerland. (B) Morphology of magnetic hydrogels. Reprinted with permission from ref. 108. Copyright 2024 Springer Japan KK. (C) Schematic of the preparation method of SF/HAP composite hydrogels. Reprinted with permission from ref. 109. Copyright 2017 AAAS Science Partner Journal Program. (D) Schematic of Ag@MSN-BMP-2/SiIMA hydrogel to promote the healing of tissue defects. Reprinted with permission from ref. 110. Copyright 2023 Oxford University Press.



and then Icariin Icariin was loaded into mHNT as a bone inducer to produce a sustained drug release system. In addition, nanocomposite chitosan/mHNTs hydrogels were prepared by sol-gel transformation, which reduced the gelation time and temperature and enhanced the mechanical strength of the resulting scaffolds, and MSCs were encapsulated into the hydrogels; *in vitro* viability assays showed the scaffolds to be biocompatible. In addition, mHNT embedded in the scaffolds led to enhanced proliferation and bone differentiation of the encapsulated cells.<sup>116</sup> The nanocomposite of polycaprolactone-polyethylene glycol-polycaprolactone/gelatin and nanotubes (PCEC/Gel/HNT) promotes stem cell (hDPSC) differentiation. The presence of nanotubes increases the surface and its roughness, facilitating scaffold-cell interaction.<sup>117</sup> It is important to note that there may be the possibility that the addition of functionalized carbon nanotubes to composite hydrogels yields two competing effects. The addition of carbon nanotubes increases the energy storage modulus of the composite hydrogel since the stiffness of the carbon nanotubes is much greater than

the stiffness of the matrix. By contrast, when the concentration of carbon nanotubes increases, the abundance of functional groups in the composite hydrogel prevents the rapid and complete cross-linking of the polymer chains, while the slow and incomplete cross-linking of the hydrogel network leads to an increase in the gelation time and decrease in the storage modulus. Therefore, larger carbon nanotube concentrations do not necessarily lead to an increase in storage modulus (Fig. 3).<sup>118</sup>

#### 4.4 Microsphere composite hydrogel scaffolds

Polymer microspheres have high potential for tissue engineering and biomaterial applications and can be used to synergistically repair and rebuild tissues by encapsulating cells, cytokines, and drugs. Alternatively, microsphere composite hydrogel scaffolds refer to a material constructed by compositing microspheres with a hydrogel material, which has better organoplastic properties and can be prepared into scaffolds with different shapes and structures.<sup>119</sup> Microsphere composite gel scaffolds can improve the stability, bioactivity and

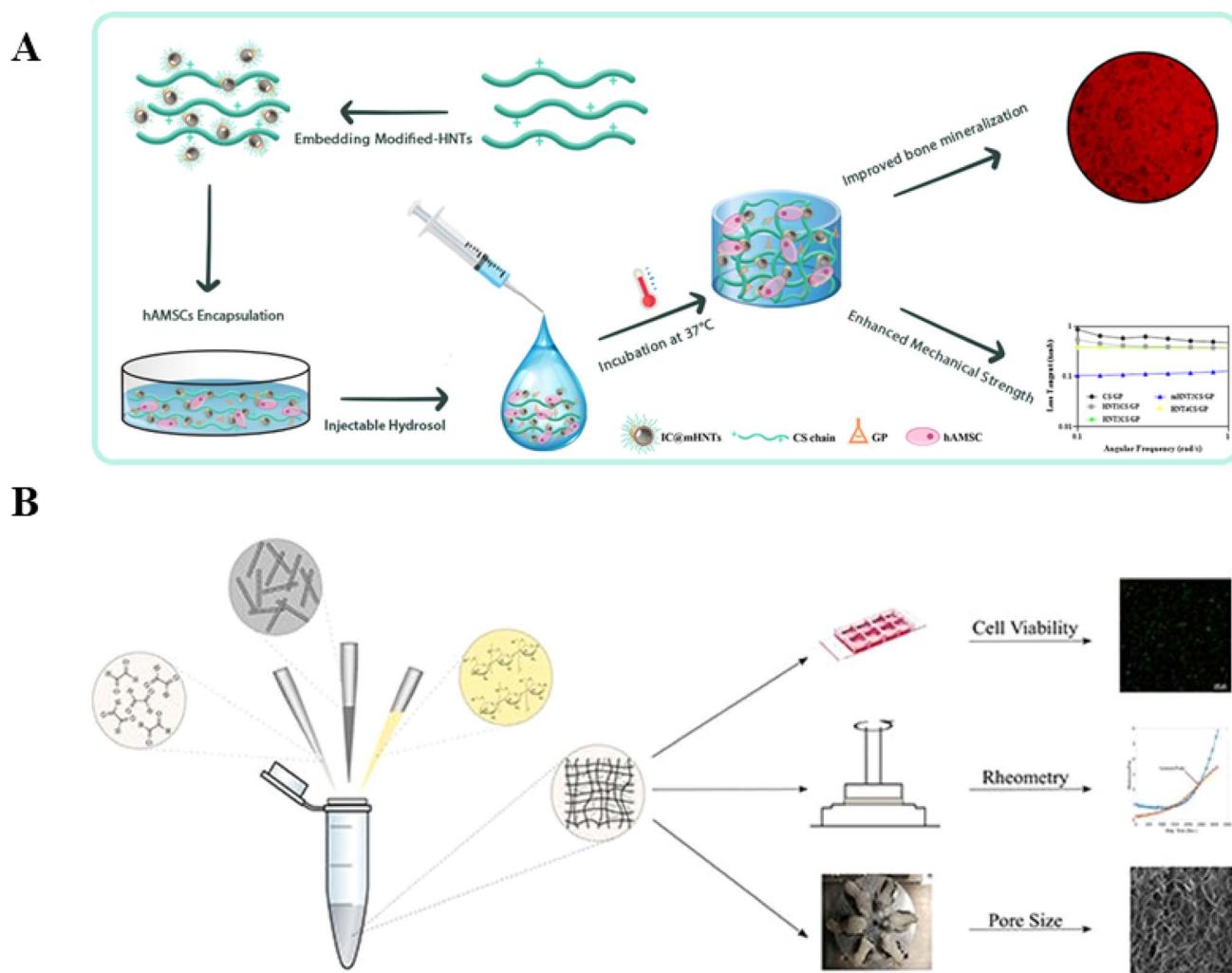


Fig. 3 Application of nanotube composite hydrogel scaffolds. (A) Injectable chitosan hydrogel embedding modified halloysite nanotubes for bone tissue engineering. Reprinted with permission from ref. 116. Copyright 2021 Elsevier Ltd. (B) Nanotube composite hydrogel scaffolds. Reprinted with permission from ref. 118. Copyright 2019 Mater Sci Eng C Mater Biol Appl.



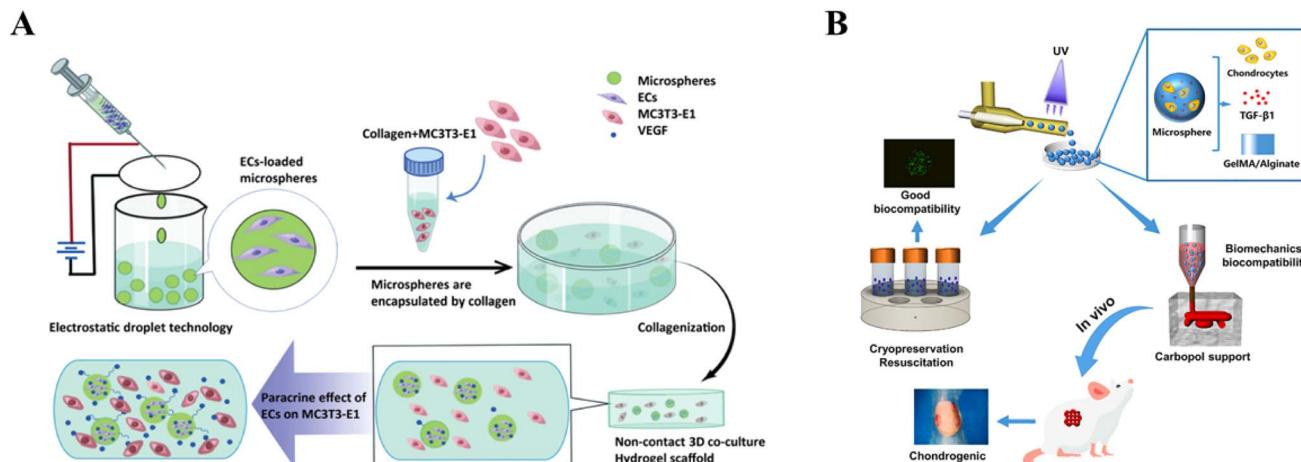


Fig. 4 Application of microsphere composite hydrogel scaffolds. (A) Indirect co-culture of type I collagen composite hydrogel scaffolds loaded with calcium alginate microspheres. Reprinted with permission from ref. 127. Copyright 2024 PLOS. (B) Microsphere composite hydrogel scaffolds for articular cartilage repair. Reprinted with permission from ref. 128. Copyright 2023 Elsevier Ltd.

biocompatibility of cells and factors by controlling the microsphere size, porosity, and surface reaction structure.<sup>120</sup> Microspheres with certain strength can be used as rigid anchors in soft hydrogels to form more physical cross-linking points, which can further increase the mechanical strength of the whole system and solve the problems of fragility or breakage of hydrogels in biomedical applications.<sup>121</sup> These physical cross-linking points can further increase the internal porosity of the hydrogel, thus improving the affinity of the hydrogel for the cells and enabling the cells to stretch in the 3D hydrogel microenvironment.<sup>122</sup> The unique surface topology of microspheres dispersed in hydrogels can increase cell affinity; the spheres facilitate cell-cell interactions but lack initial adhesion to natural or artificial ECMs, whereas hydrogels are ECM-like structures that promote initial cell adhesion. Therefore, microspheres not only improve the internal structure of hydrogels but also provide more internal space for cell proliferation and migration, thus providing favorable conditions for cellular respiration and transport of nutrients and metabolic wastes.<sup>123</sup> Polymer microspheres have good mechanical properties, biocompatibility, and degradability but poor hydrophilicity.<sup>124</sup> Lin *et al.* used PLGA microspheres carrying growth factors in combination with methoxypoly(ethylene glycol)-poly(poly(alanine)) (mPA) hydrogel, and results showed the ability to provide excellent 3D microenvironment for the promotion of cartilage-forming phenotypes. This synthesized composite hydrogel system was also able to regulate chondrocyte biosynthesis and differentiation activity.<sup>88</sup> Ji *et al.* newly developed a composite scaffold consisting of porous chitosan (CS) microspheres and hydroxypropyl chitin (HPC) hydrogel, in which dimethyloxalylglycine (DMOG) was encapsulated in a heat-sensitive HPC hydrogel (HD), and tyrosine (KGN) coupled to porous CS microspheres (CSK-PMS) was employed as a controlled drug delivery system that can effectively create the M2 macrophage microenvironment and coordinate osteochondral regeneration.<sup>125</sup> However, microspheres prepared by current techniques have a single internal and external structure,

high dispersion and low yield, which can affect cell and drug delivery scalability and biocompatibility<sup>126</sup> (Fig. 4).

## 5. Preparation of hydrogel composite scaffolds

Hydrogel composites have attracted widespread attention due to their superior properties. The preparation of hydrogel composite scaffold materials with good biocompatibility, tunable degradability, and mechanical strength to improve their efficacy in clinical applications has become a major goal. The selection of crosslinking and fabrication strategies for hydrogel scaffolds must align with target tissue requirements, balancing mechanical performance, biocompatibility, and structural fidelity. Innovations in hybrid techniques, dynamic materials, and precision manufacturing will accelerate the translation of hydrogel-based scaffolds into clinically viable multifunctional platforms for complex tissue regeneration (Table 3). Herein, we present the main preparation methods of hydrogel composite scaffolds.

### 5.1 Physical cross-linking

Physical cross-linking method is one of the commonly used techniques to prepare polymer scaffolds.<sup>127</sup> Physical hydrogels are formed utilizing physical interactions, which involves non-covalent interactions (e.g., hydrogen bonds, ionic bonds), to allow polymers to form an ordered network structure. Compared to chemical cross-linking, physically cross-linked hydrogels usually have better solubility and can be released in a controlled manner under changing conditions such as temperature or pH. However, the prepared physical hydrogels usually have poor mechanical properties and structural stability, which are the main limitations of this crosslinking method.<sup>128,129</sup> There are several common physical cross-linking methods, including freeze-thawing, ionic interactions, and hydrogen bonding.<sup>130-132</sup> Freeze-thawing method forms





Table 3 Comparison of performance of hydrogel composite scaffolds' preparation methods

Parameter	Physical cross-linking	Chemical cross-linking	Photocrosslinking	3D printing
Mechanical strength	Low (kPa range)	High (MPa range)	Moderate-high (process-dependent)	Moderate-high (process-dependent)
Biocompatibility	Excellent	Moderate (post-purification)	Good (low phototoxicity)	Moderate (process-dependent)
Degradation control	Short-term (days-weeks)	Long-term (months-years)	Tunable (covalent + photolysis)	Programmable (multi-material)
Structural complexity	Low (homogeneous)	Moderate (pre-molded)	High (light-patterned)	Extremely high (3D microarchitecture)
Clinical potential	Injectable fillers	Long-term implants	Minimally invasive <i>in situ</i> gels	Customized tissue substitutes

microcrystalline structures through multiple freeze–thaw cycles, which reduces the gaps between polymer chains, increases the concentration of polymers, and forms interconnected network structures, with no toxic effect on cells and no effect on the biocompatibility and biodegradability of polymerized hydrogels.<sup>133</sup> Nie *et al.* used cyclic freeze–thawing to prepare chitosan/gelatin hydrogels with biphasic calcium phosphate nanoparticles (BCP-NPs) as scaffolds for bone tissue engineering (CGB); the prepared scaffolds showed good cytocompatibility and induced bone regeneration in bone marrow mesenchymal stem cell (BMSC) culture.<sup>134</sup> Ionic interaction, alternatively, is a method of gelling the polyelectrolyte solution by the addition of divalent and trivalent ions, in which multivalent ions form crosslinked structures with oppositely charged groups in the polymer.<sup>132</sup> Hydrogen bonding is an important physical interaction that can lead to the formation of tertiary and secondary structures in hydrogels. This interaction is based on the degree of protonation present in the chemical environment and protonation of polar functional groups. Through hydrogen bonding interactions, soft materials can be endowed with self-healing properties. Physical cross-linking networks of composite hydrogels created through intramolecular or intermolecular hydrogen bonding increase the heat resistance of the composite hydrogels and decrease the rate of swelling and biodegradation.<sup>135</sup> The main drawback of these physical cross-linking methods is the lack of sufficient strength and mechanical stability (Fig. 5).

## 5.2 Chemical cross-linking

Chemically crosslinked hydrogels are covalently crosslinked by the addition of chemical reagents, which endows the hydrogel higher mechanical strength. In contrast to physical cross-linking, chemically cross-linked hydrogels are formed by irreversible covalent bonding, which are known as permanent hydrogels, and their swelling properties depend mainly on the concentration of the cross-linking agent.<sup>136</sup> Free radical polymerization is one of the methods that often uses vinyl monomers or their functionalized forms with free radical initiators and cross-linking agents added. The advantage of this method is that a wide range of monomers can be used for the synthesis of biologically active hydrogels, but it requires photo-stimulation or thermal stimulation to initiate cross-linking, which can cause cytotoxicity.<sup>137</sup> Tanasa *et al.* prepared nanocomposite hydrogels that stimulate soft tissue regeneration by the free radical polymerization of magnetite nanoparticles modified by acrylamide monomers and double bonds.<sup>138</sup> Schiff base reaction is used to form hydrogels through the reaction between amino and aldehyde groups, and the reaction of natural polymers with natural or synthetic polymers containing amino groups produces hydrogels with high reaction rates. Alginate/gelatin hydrogel scaffolds embedded in chitosan microspheres containing curcumin were formed *in situ* by the Schiff base reaction for bone tissue regeneration.<sup>139</sup> The Diels–Alder reaction, alkyne–azide cycloaddition reaction, and thiophene reaction are also commonly used for the preparation of composite scaffolds in hydrogels, but their slow kinetics have

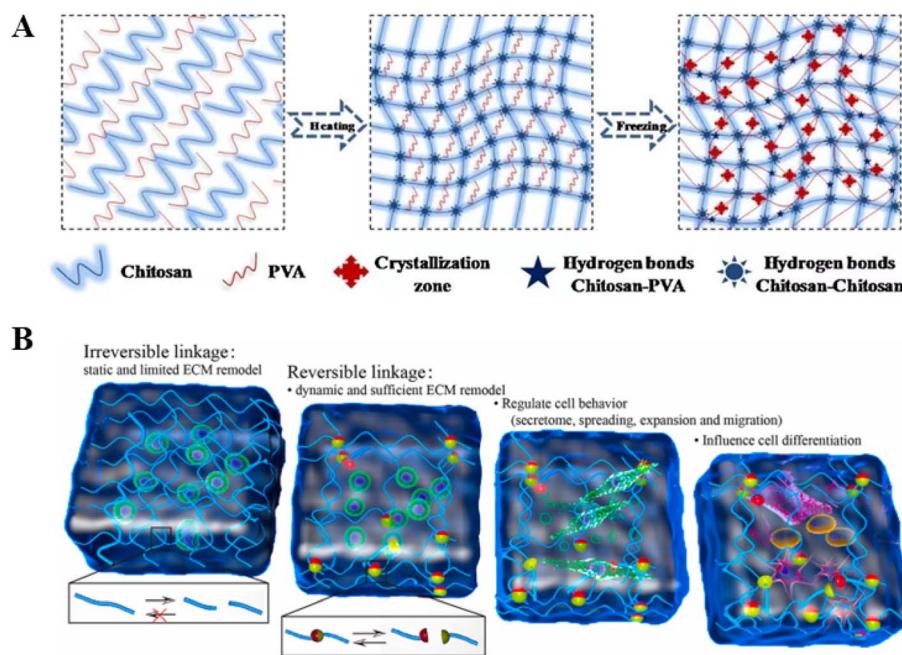


Fig. 5 Physical cross-linking. (A) Physical cross-linking to construct chitosan/PVA bi-network hydrogels. Reprinted with permission from ref. 49. Copyright 2002 Elsevier Ltd. (B) Reversible linkages based on supramolecular interactions and dynamic covalent bonds. Reprinted with permission from ref. 132. Copyright 2020 KeAi Publishing.

hindered their development. Enzymatic reactions can be used to prepare polymers containing enzyme-responsive regions such as tyrosine, tyramine, aminophenol, and dopamine, and rapid *in situ* gelation occurs during oxidation using hydrogen peroxide. Commonly used cross-linking agents include glutaraldehyde (GA), formaldehyde (FA), curcumin, and genipin (GE).<sup>140</sup> However, the use of cross-linking agents may cause toxicity, and to overcome the side effects of polymers and

improve stability, the use of non-cytotoxic natural cross-linking agents may be an option (Fig. 6).<sup>141</sup>

### 5.3 Photocrosslinking

Photocrosslinking is generally considered a form of chemical cross-linking, except in specific cases where physical cross-linking may occur. It is based on a free radical polymerization reaction initiated by a photoinitiator, which crosslinks polymer

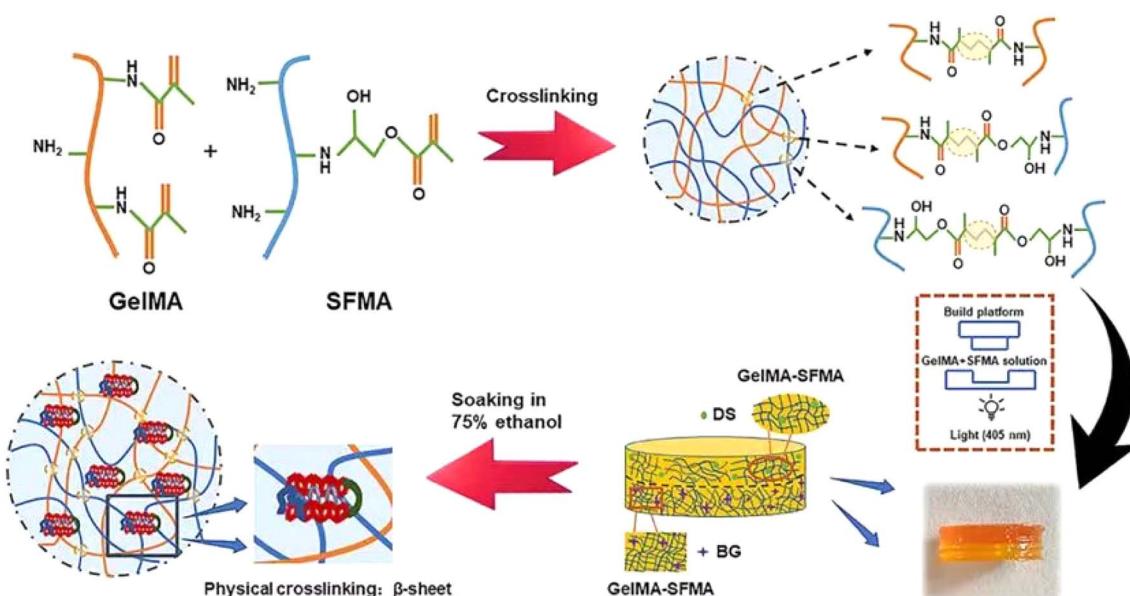


Fig. 6 Chemical cross-linking preparation of GelMA-SFMA hydrogels. Reprinted with permission from ref. 141. Copyright 2024 Springer Nature.

molecules together to form a 3D network structure by means of a crosslinking agent, capable of controlling the position and timing of the crosslinks in a physiological environment.<sup>142</sup> Photocrosslinking consists of initiation, propagation and termination steps performed under UV-visible light irradiation and photoinitiators.<sup>143,144</sup> Free radicals dissociate from the photoinitiator upon irradiation and attack the vinyl group on the macromolecular precursor, and light irradiation creates covalent bonds that crosslink the hydrogel network within seconds to minutes. Selection of suitable photoinitiators can inhibit the undesirable cytotoxic effects of cell encapsulation.<sup>145</sup> A number of key characteristics need to be considered when selecting a photoinitiator, such as the ability to generate free radicals, absorption spectrum, water solubility, molar extinction coefficient, and stability. Visible photoinitiators are relatively less cytotoxic and have high water solubility compared to UV photoinitiators.<sup>146</sup> Paek *et al.* developed a GelMA photocrosslinking system based on a pore plate with tunable hydrogel mechanical properties to modulate PTH-mediated osteogenic fate. In response to PTH, hMSCs in a high stiffness microenvironment upregulated osteogenic differentiation.<sup>147</sup> Another study demonstrated the visible light-induced photocrosslinking of methacrylate- $\kappa$ -carrageenan (MA- $\kappa$ -CA) mixed with bioactive silica nanoparticles (BSNPs) for the fabrication of 3D composite hydrogels using digital light processing (DLP) printing, which showed high cellular viability, no cytotoxicity, and significantly enhanced osteogenic differentiation.<sup>148</sup> Photocrosslinking requires the addition of photosensitizers to activate the crosslinking reaction; however, for some sensitive cells or biomolecules, using photosensitizers may adversely affect their activity

or function. Photocrosslinking requires the use of specific wavelengths and intensities of light to activate the cross-linking reaction, and if the light conditions are not appropriate or stable, it may result in suboptimal cross-linking (Fig. 7).<sup>149</sup>

#### 5.4 3D printing

In the preparation process of hydrogel composite scaffolds, 3D printing technology can improve the production efficiency, consistency and reproducibility of the prepared hydrogel composite scaffolds owing to advantages such as printing design refinement, accelerated production speed, reduced manual operation, and improved precision of preparation, which cannot be achieved by the traditional manual production methods.<sup>150</sup> The data associated with 3D printing technology comes from 3D model data based on computer design. After slicing the 3D model into 2D code information, 3D printers can recognize it. The principle of 3D printing technology is broadly defined as the layer by layer stacking of materials to achieve free-form fabrication, but there are different categories of 3D printing molding principles.<sup>151</sup> Unlike traditional hydrogel scaffolds, 3D printing technology allows the preparation of a variety of complex and fine structures, such as nano or particle-sized channels and pores, for natural cell adhesion and differentiation of cells through hydrogel composite scaffolds, accelerating bio-integration of real tissue growth and cell transplantation. In addition, 3D printing technology can control the porosity of the scaffolds, pore size and orientation, *etc.*, to achieve the creation of an ideal acid-base balance, cell supply, and generative growth

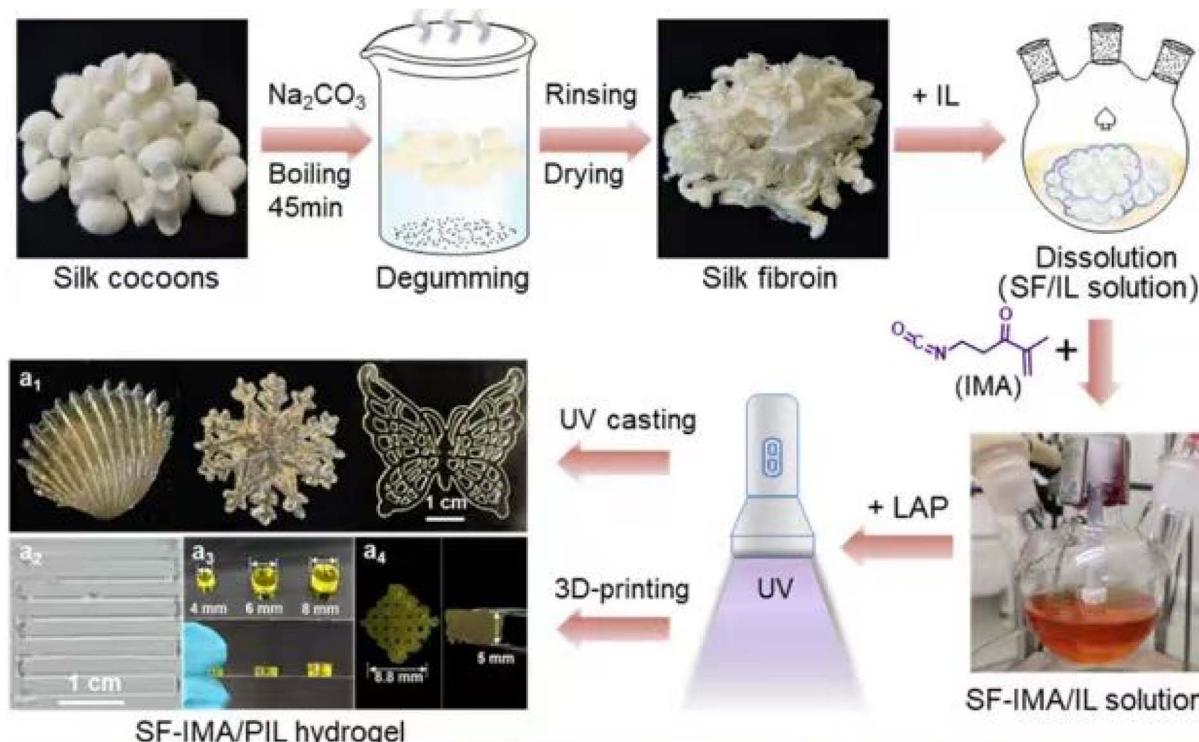


Fig. 7 SF-IMA/PIL hydrogel photocrosslinking preparation. Reprinted with permission from ref. 149. Copyright 2024 American Chemical Society.



environment, and to improve the efficiency of tissue regeneration and success rate of cell regeneration (Table 4).<sup>161</sup> For example, Jiang *et al.* used 3D bioprinting to construct scaffolds containing mesenchymal stem cells (MSCs) in GelMA/graphene oxide (GO) complexes and found that GO enhanced cell proliferation, but it had no significant effect on cell viability. *In vitro* experiments showed that GO promoted substance-cell interactions and expression of osteogenesis-related genes. *In vivo* experiments showed that GO decreased degradation time of the material and increased deposition of calcium nodules, and incorporation of GO created a suitable microenvironment to promote the differentiation of exogenous MSCs loaded *in vitro* and *in vivo* for bone defects repair compared to pure GelMA.<sup>162</sup> nHA/PLA/dECM/β-CD, synthesized by Li *et al.* through 3D printing technology, was found to be a good choice for bone defects repair. Composite scaffolds showed promise in inducing bone regeneration, preventing

infection, and promoting jaw defect repair.<sup>163</sup> Bartnikowski *et al.* prepared multilayered hydrogel composites by 3D printing, casting functionalized gelatin methacrylamide (GelMA) or GelMA with hyaluronic acid methacrylate (HAMA) on pure alginate or alginate/hydroxyapatite (HAp) composites; the incorporation of hydroxyapatite increased the elastic modulus of the printed hydrogel composites, while HAMA in the GelMA hydrogels improved cartilage formation.<sup>164</sup> Currently, the available hydrogel cross-linking methods are severely limited, with only photo- and ionic-cross-linking strategies applicable to 3D printing, and the materials and printing systems for these methods are not able to meet the stringent requirements for tissue engineering applications. Meanwhile, most hydrogel composites are simply mixed with different weight ratios of components, which leads to severe agglomeration of reinforcing materials within the hydrogel matrix and affects the material properties (Fig. 8).<sup>165,166</sup>

Table 4 3D printing to make hydrogel composites

3D printing method	Reinforcement/loading	Base materials	Performances	Application	Ref.
DIW	LAP	2-Hydroxyethyl methacrylate	Activation ( <i>e.g.</i> , protein) treatment is not required to promote robust growth compliance that directs the spatial attachment of fibroblasts (3T3) and osteoblast pre-cells, thereby cultivating the latter's ability to direct long-term bone differentiation	Tissue engineering	152
	RGD-PDL	(2-Hydroxyethyl) methacrylate (pHEMA)	Allows direct formation of cellular networks and organotypic structures	Regenerative medicine	153
	Glycidyl methacrylate	HAc	Improved mechanical properties	Tissue engineering	154
SLM	Alg-PCL	Alginate	Ideal for fast adaptation to a range of interchangeable melts and high-viscosity materials	Additive manufacturing	155
	PLA	Gelatin-chitosan	Enhance mechanical stability, biocompatibility and biological activity	Tissue engineering	156
FDM	PEEK	MeHA-HAp	Promotes the adhesion and proliferation of mesenchymal stem cells, aids in osteogenic differentiation and extracellular matrix mineralization	Biomedicine	157
	GMHA	Poly(ethylene glycol)-block-poly(β-caprolactone) scaffold (mPEG-PCL), hydroxyapatite (HAp) powder and grafting RGD peptide to the surface of the scaffold	Promotes healing of kneecap cartilage defects in rabbits	Tissue engineering	158
	Curcumin	Gelatin methacrylate (GelMA)/chitosan nanoparticles (CSNPs)	Effectively protects against gram-positive and gram-negative bacteria, promotes cell proliferation and reduces microbial infections in wounds	Wound healing	159
Extrusion bioprinting	hpcECM	SF and G-TA	It has improved and tunable biomechanical properties for encapsulated cells as well as high biological activity	Soft tissue engineering	160



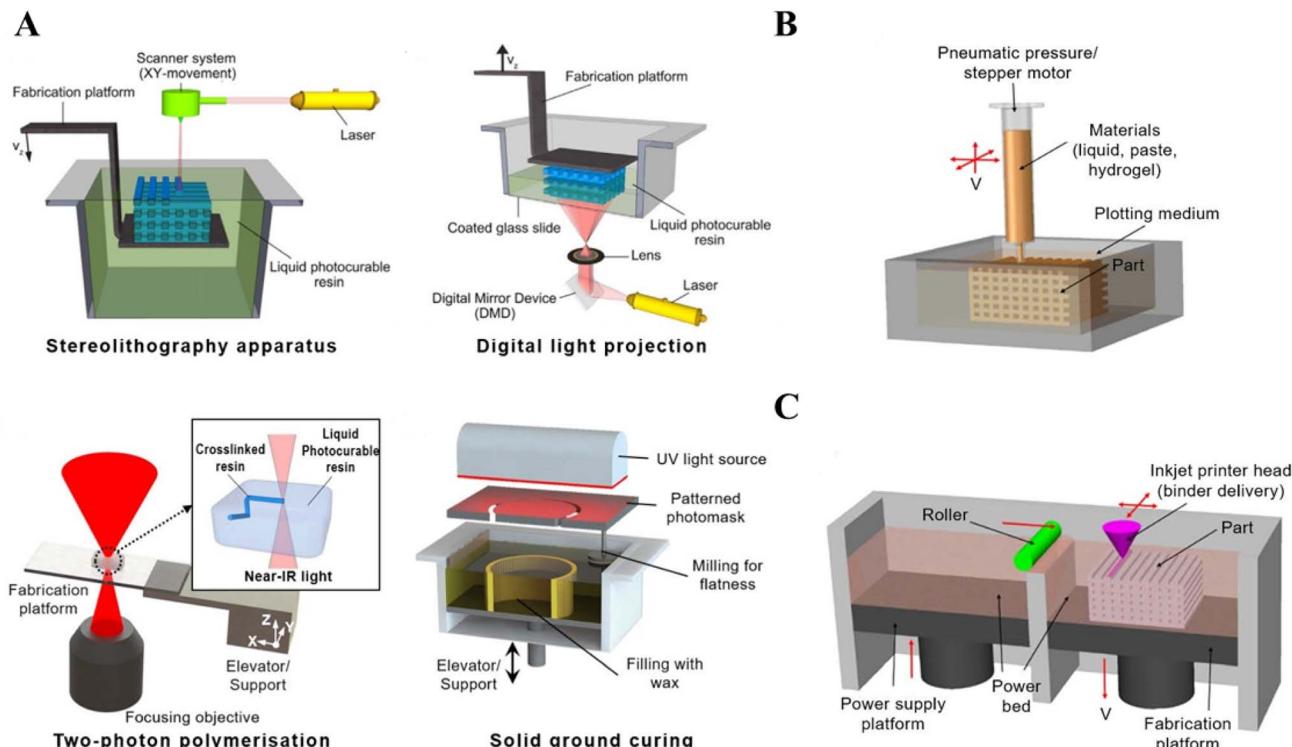


Fig. 8 3D printing technology for hydrogel composites. (A) Laser-based hydrogel 3D printing system. Reprinted with permission from ref. 165. Copyright 2012 Elsevier Ltd. (B) Nozzle-based hydrogel 3D printing system. Reprinted with permission from ref. 166. Copyright 2017 Elsevier Ltd. (C) Inkjet printer-based hydrogel 3D printing system. Reprinted with permission from ref. 166. Copyright 2017 Elsevier Ltd.

## 6. Application of hydrogel composite scaffold in knee cartilage defects

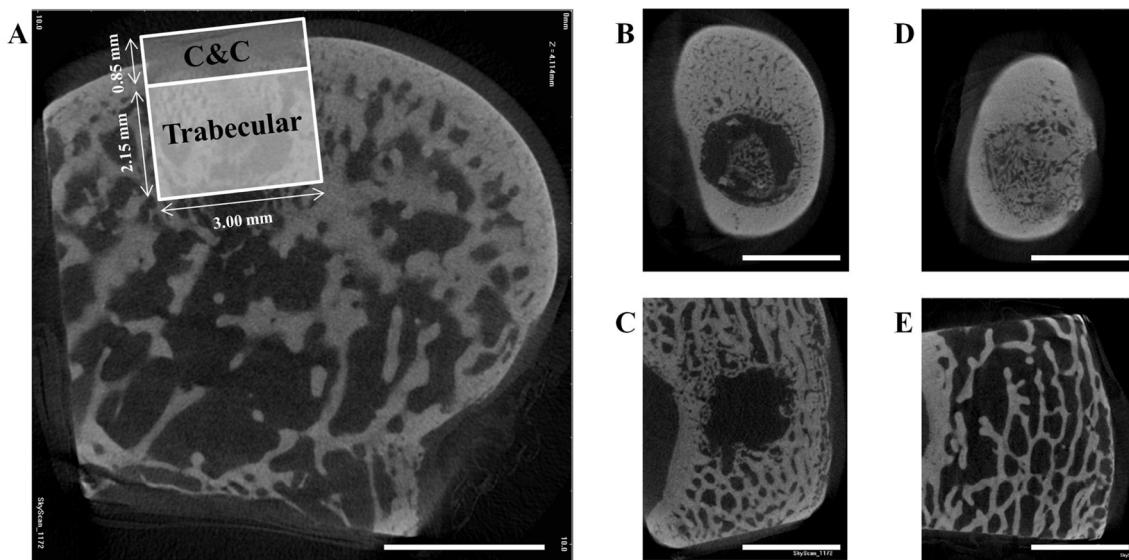
When patients' knee cartilage is damaged, traditional treatment mainly relies on the repair and regeneration of their own cartilage. However, the limited ability to repair cartilage defects often leads to aggravation of the condition and even requires knee replacement surgery. The hydrogel composite scaffold material can provide a suitable three-dimensional scaffold structure for chondrocyte settlement and tissue regeneration as it can be tightly integrated with the damaged cartilage area, acting as a bridge so that cells can grow and settle in the defective area and create a microenvironment conducive to the regeneration of chondrocytes. The composite scaffold is able to degrade and transform into the extracellular matrix on its own, forming a complete group structure with the cells and providing good mechanical support. In clinical application, it can be customized according to the shape, size and location of the patient's knee cartilage defect to better fit the defect site and improve the accuracy and completeness of repair. Some hydrogel composite stents can be implanted into the body through minimally invasive injections to reduce surgical trauma and complications, shorten the patient's recovery time, and improve treatment experience. It can also be combined with existing knee cartilage repair technologies such as arthroscopic technology and microfracture technology to give full play to their respective advantages and further improve the

treatment effect. In recent years, an increasing number of clinical studies have been conducted to apply hydrogel composite scaffolds in the treatment of knee cartilage defects.

### 6.1 Medial femoral condyle

Cartilage lesions of the medial femoral condyle are the most common knee joint site other than the femoral patellofemoral joint. Cartilage injuries frequently occur in the main stress zone of the medial femoral condyle due to mechanical stress and kinematic orientation.<sup>167</sup> Dashtdar *et al.* formed medial femoral condyle defects in both knees of 24 adult New Zealand white rabbits, transplanted a novel polyvinyl alcohol (PVA)-chitosan composite hydrogel-mesenchymal stem cell (MSC) and evaluated cartilage repair at six months. Significant tissue repair was observed on treatment with PVA chitosan-MSC. In addition, the saffron O staining and glycosaminoglycan (GAG) content of the MSC-treated group was significantly higher than that of the scaffold-only or untreated control group, suggesting that PVA-chitosan composite hydrogel scaffolds can be used as a carrier of MSC for the treatment of cartilage defects.<sup>168</sup> Lu *et al.* investigated the use of biodegradable hydrogel composite scaffolds based on macro-oligomer (poly(ethylene glycol)) fumarate (OPF) in medial femoral condylar osteochondral defects, in which three different scaffolds were implanted to provide growth factors for repairing osteochondral tissue in a rabbit model. The bilayer OPF hydrogel composite scaffold showed potential as a spatially-guided multiple growth factor release carrier for

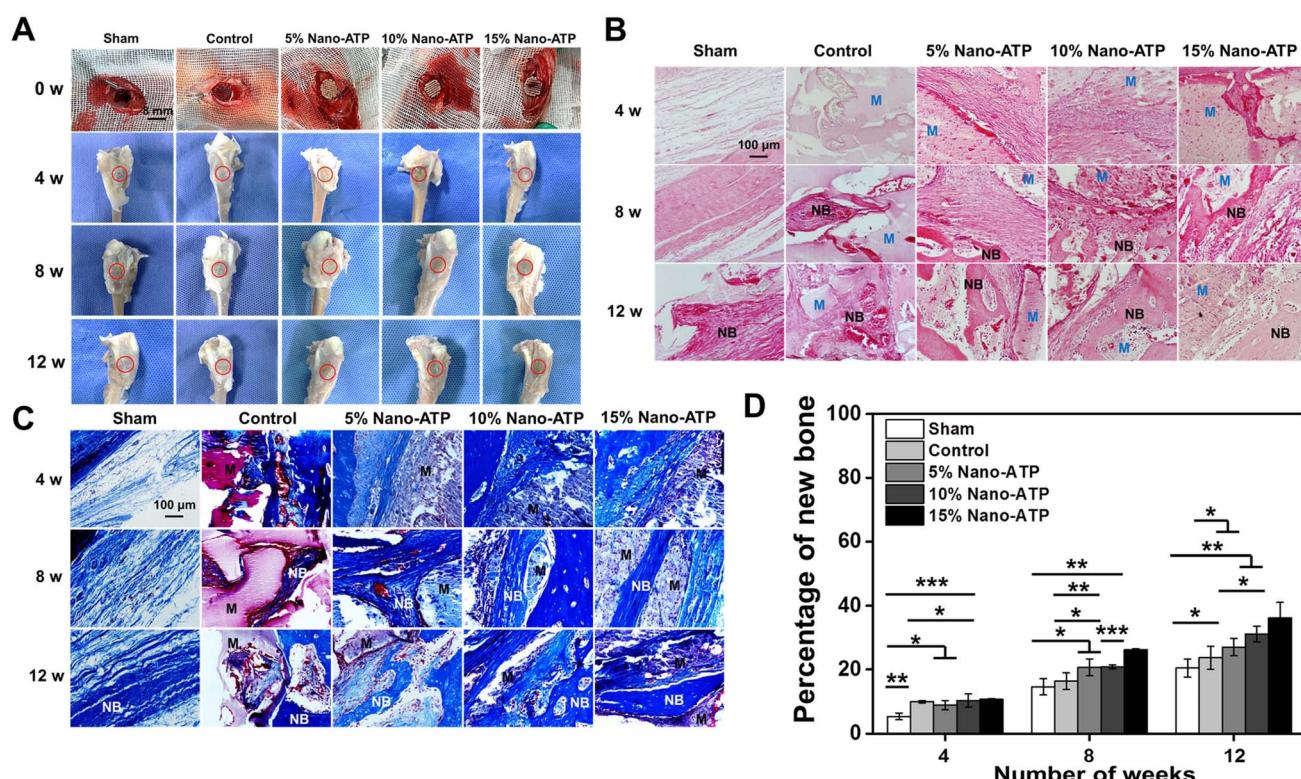




**Fig. 9** CT analysis of the cartilage repair effect of a hydrogel composite scaffold loaded with growth factors on medial femoral condylar defects. (A) Cross section of osteochondral defect, cortex. (B) Trabeculae. (C) Cortical area after six weeks of treatment. (D) Trabeculae after six weeks of treatment. (E) Section of the sample after 12 weeks of treatment. Reprinted with permission from ref. 169. Copyright 2014 Elsevier Ltd.

osteochondral tissue repair.<sup>169</sup> Using a goat model, autologous chondrocytes immobilized in MPEG-PLGA scaffolds *via* fibrin hydrogel produced a 6 mm round, full-length cartilage defect in

both medial femoral condyles, which was evaluated for cartilage repair potential and showed favorable cartilage repair response<sup>170</sup> (Fig. 9). Various approaches utilizing advanced



**Fig. 10** Histological analysis of gel/SA/nano-ATP composite hydrogel scaffolds in tibial plateau defects in rabbits 4–12 weeks after implantation. (A) Photographs of the five experimental groups during surgery and 4–12 weeks postoperatively. (B) Observation of H&E staining. (C) Observation of the new bone formed in the composite hydrogel. (D) Quantitative analysis of Masson staining. Reprinted with permission from ref. 171. Copyright 2021 Dove Press.

hydrogel scaffolds and stem cell therapies demonstrate promise for repairing cartilage lesions in the medial femoral condyle. These advancements support the potential for developing effective treatments for cartilage defects, although further research is needed to optimize these techniques for clinical application.

## 6.2 Tibial plateau

There are few reports on hydrogel composite scaffolds for the treatment of tibial plateau defects. In one study, sodium alginate (SA)/gelatin (Gel) hydrogel scaffolds doped with different contents of nano-gravite were prepared by 3D printing

technology, and their surface microstructure, hydrophilicity, and mechanical properties were evaluated. In addition, mouse bone marrow-derived mesenchymal stem cells (BMSCs) were cultured with the composite hydrogels *in vitro* and evaluated for proliferation and osteoblast differentiation, while a rabbit tibial plateau defect model was used to assess the osteogenic potential of the composite hydrogels *in vivo*. Results showed that the Gel/SA/nano-ATP composite hydrogels exhibited better mechanical properties and printability when the nano-ATP content was increased. They showed excellent bioactivity, and a significant mineralization effect was observed on the surface after incubation in simulated body fluid (SBF) for 14 d. The Gel/SA/nano-ATP composite hydrogel also showed good

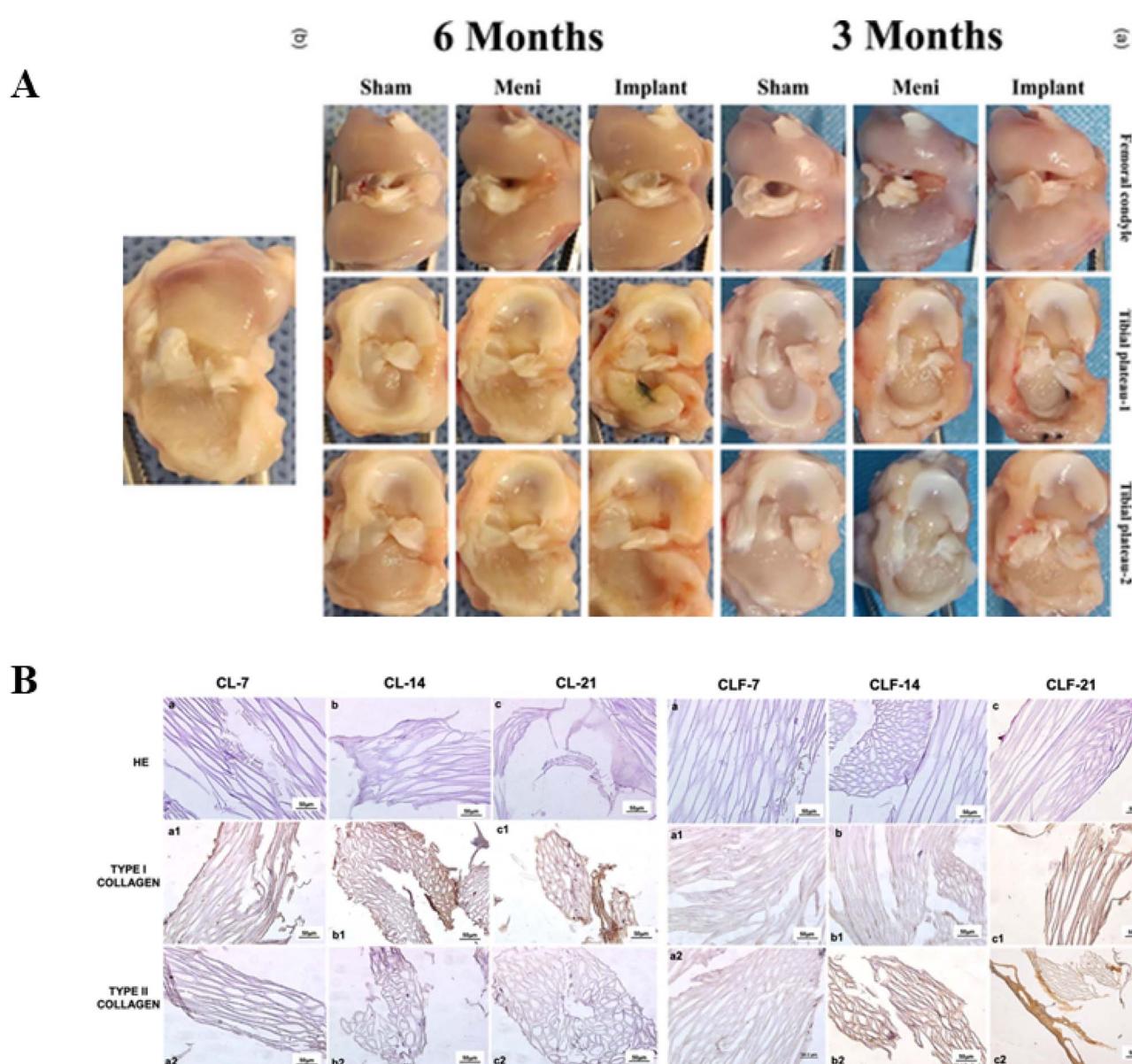


Fig. 11 Hydrogel composite scaffold repairs meniscus. (A) Examining the gross changes in articular cartilage after the replacement of the meniscus with a PG-Pg scaffold. Reprinted with permission from ref. 172. 2024 ESSKA a.s.b.l. (B) Histological sections of hydrogel composite scaffolds loaded with mesenchymal cells stained for H&E and type I collagen/type II collagen. Reprinted with permission from ref. 173. Copyright 2022 Elsevier Ltd.



biocompatibility and could effectively promote bone regeneration *in vivo* (Fig. 10).<sup>171</sup>

### 6.3 Meniscus

The meniscus is a fibrocartilaginous tissue that is important for the stability of the knee joint. However, it has low self-healing ability; thus, damage to it always leads to articular cartilage degeneration. Li *et al.* performed total meniscus replacement in a rabbit model using a 3D printed network hydrogel composite scaffold to investigate whether hydrogel composite scaffolds can prevent articular cartilage degeneration, promote tissue degeneration, and to explore its mechanical properties at three months and six months postoperatively. Results showed that the hydrogel composite scaffold slowed down articular cartilage degeneration, promoted tissue regeneration, and facilitated articular cartilage regeneration.<sup>172</sup> Baysan *et al.* fabricated a new type of meniscus hydrogel composite scaffold made of chitosan, loofah mat, and PHBV nanofibers, and *in vitro* analyses showed no cytotoxic effects, allowing cells to attach, proliferate, and migrate inside the scaffold, making it a promising meniscus tissue engineering material.<sup>173</sup> Chitosan-collagen hydrogel composite scaffolds consisting of 3D-printed polylactic acid (PLA) struts and nanofibrous cellulose showed no cytotoxic effects on rabbit mesenchymal stem cells, enabling cells to attach, proliferate, and migrate through the inner regions of the scaffolds, which can be used for meniscal cartilage tissue engineering<sup>174</sup> (Fig. 11).

### 6.4 Others

A composite scaffold for cartilage defect repair was developed by incorporating a carbon nanotube-doped octapeptide hydrogel into a 3D-printed PCL scaffold (FEK/C-S), where CNTs were

fabricated in a network of a previously introduced non-immunogenic octapeptide (FEFEFKFK) to form a novel hydrogel denoted as FEK/C. Then, FEK/C was applied to 3D-printed PCL scaffolds to form a composite scaffold denoted as FEK/C-S. The composite scaffolds promoted cartilage and subchondral bone regeneration in a rabbit knee defect model.<sup>175</sup> Long *et al.* developed an ultrasonication-induced silica-gel-collagen composite hydrogel (COL + S(S)), which demonstrated excellent stability during cell culture. It promoted SOX9 gene expression at an early stage and sulfated glycosaminoglycan (sGAG) deposition in the absence of any exogenous growth factors. In addition, well-integrated articular hyaline cartilage, very similar to natural articular cartilage, was demonstrated in a rabbit knee defect model.<sup>176</sup> Wu *et al.* developed a TGF- $\beta$ 3-loaded TGF- $\beta$ 3-containing methacrylate sericin-protein sealant (Sil-MA), which is biocompatible and has good adhesive properties to promote chondrocyte migration and differentiation. Importantly, this TGF- $\beta$ 3-loaded Sil-MA hydrogel bridges the gap between the cartilage layer of the scaffold and the surrounding cartilage, which then guides the growth of new cartilage toward the surrounding natural cartilage and replaces the degraded cartilage layer in the early stages of knee repair. Using this composite, osteochondral regeneration and superior lateral integration were achieved *in vivo*.<sup>177</sup> Li evaluated the ability of filipin protein (SF) hydrogel scaffolds doped with chitosan (CS) nanoparticles (NPs) to repair cartilage defects in the knee joint. The developed TGF- $\beta$  1@CS/BMP-2@SF hydrogel promoted the chondrogenic capacity of BMSCs *in vivo* and *in vitro* by releasing TGF- $\beta$ 1 and BMP-2.<sup>178</sup> In another study, a solution containing TGF- $\beta$ 1 was photo-crosslinked to form a hydrogel layer immobilized on the upper side of an RP scaffold. Using a scaffold designed for fused

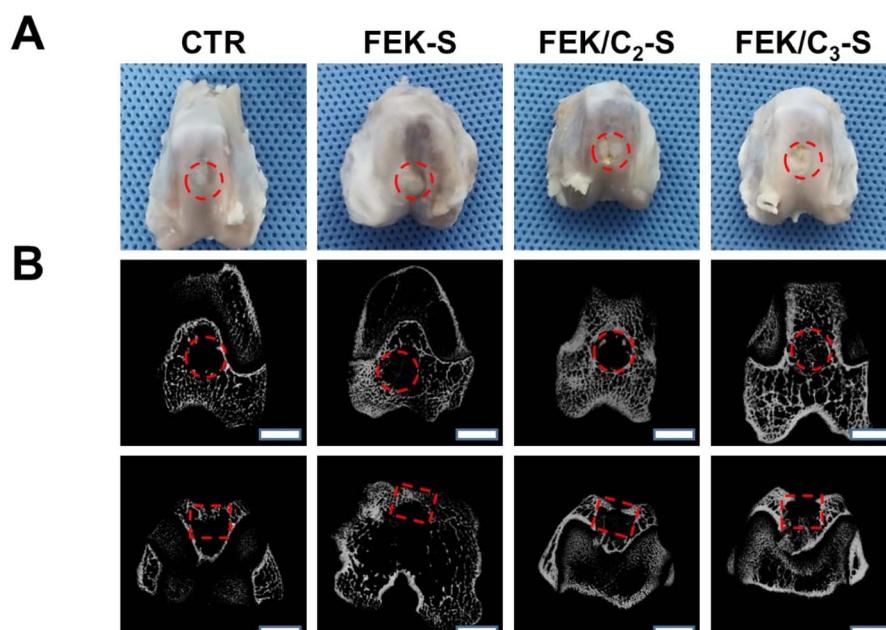


Fig. 12 Effect of composite scaffolds on the repair of articular cartilage and subchondral bone defects in rabbits. (A) Images of rabbit knee joints after articular defect and scaffold implantation eight weeks postoperatively. (B) CT scans, defective areas are shown as red circles or red squares. Reprinted with permission from ref. 175. Copyright 2023 MDPI, Basel, Switzerland.



deposition modeling, the damaged articular cartilage and subchondral bone were repaired and regenerated in an *in vivo* trial in the knee of a Lanyu miniature pig over a 12 month period.<sup>179</sup> In another study, osteochondral regeneration induced by TGF- $\beta$ -loaded photocrosslinked hyaluronic acid hydrogel infiltrated in a composite scaffold fabricated by the fused deposition of hydroxyapatite and poly(ethylene glycol)-block-poly( $\epsilon$ -caprolactone) was investigated in an *in vivo* trial on rabbit knees; the histological sections showed that 12 weeks after implantation of the TGF- $\beta$ 1-loaded hydrogel and scaffolds, the bone and cartilage defects produced in the knee joints were completely healed and the regenerated cartilage was hyaline cartilage<sup>158</sup> (Fig. 12).

## 7. Conclusion

In recent years, research on hydrogel composite scaffolds in regenerative medicine and tissue engineering has achieved numerous positive outcomes. Although current research on knee cartilage defects is somewhat limited, it has exhibited great potential. Hydrogel composite scaffolds possess excellent biocompatibility and can integrate seamlessly with surrounding tissues, thus providing an ideal growth environment for promoting the regeneration and repair of chondrocytes. Treating knee joint cartilage defects with hydrogel composite scaffolds can significantly relieve patient pain and functional limitations. Moreover, hydrogel composite scaffolds have adjustable release properties. They can release beneficial cytokines and growth factors, which stimulate the proliferation and differentiation of chondrocytes, thereby promoting cartilage regeneration. Additionally, these scaffolds can serve as carriers for introducing stem cells or genes into cartilage defects, further accelerating the cartilage repair process.

However, current research also confronts several challenges and limitations. Firstly, the long-term efficacy and safety of hydrogel composite scaffolds require further evaluation. Secondly, the preparation and manipulation techniques of these scaffolds need to be refined to enhance preparation efficiency and controllability. Thirdly, more clinical studies are essential to validate the efficacy of hydrogel composite scaffolds in treating different types and degrees of cartilage defects.

To address these issues, researchers can focus on the following emerging trends. To improve the mechanical properties, exploring novel composite materials and cross-linking methods can enhance the strength and durability of hydrogel composite scaffolds, enabling them to better withstand the mechanical forces within the knee joint. For more precise drug release, advanced drug-loading and release-control technologies, such as smart hydrogels that respond to specific physiological signals (e.g., pH, temperature, or enzyme activity), can be developed, ensuring that growth factors and therapeutic agents are released at the optimal time and dosage to optimize cartilage repair. Integrating stem cell therapies with hydrogel composite scaffolds also holds great promise. By precisely regulating the microenvironment provided by the scaffolds, stem cells can be more effectively guided to differentiate into chondrocytes, thus enhancing cartilage regeneration. For instance, incorporating signaling molecules or extracellular

matrix components into the scaffolds can promote the differentiation of stem cells into chondrocytes. In addition, with the assistance of advanced technologies such as bioprinting, more precise and personalized scaffolds can be fabricated to meet the specific requirements of different patients and cartilage defect types. This personalized approach is likely to improve treatment outcomes and patient satisfaction.

In conclusion, the study of hydrogel composite scaffolds in knee cartilage defects presents a promising outlook. With continuous technological progress and in-depth research, hydrogel composite scaffolds are expected to become a crucial therapeutic tool, bringing better clinical results for patients with knee cartilage defects.

## Author contributions

Conceptualization, D. T. and H. S.; resources: D. T., H. S., C. Y. and Y. L.; writing – original draft preparation, D. T.; writing – review and editing, X. S. and S. R. All authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

The authors have no conflict of interest to declare.

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