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Historical Perspective

Injectable hydrogels: An emerging therapeutic strategy for cartilage regeneration

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ABSTRACT

The impairment of articular cartilage due to traumatic incidents or osteoarthritis has posed significant challenges for healthcare practitioners, researchers, and individuals suffering from these conditions. Due to the absence of an approved treatment strategy for the complete restoration of cartilage defects to their native state, the tissue condition often deteriorates over time, leading to osteoarthritic (OA). However, recent advancements in the field of regenerative medicine have unveiled promising prospects through the utilization of injectable hydrogels. This versatile class of biomaterials, characterized by their ability to emulate the characteristics of native articular cartilage, offers the distinct advantage of minimally invasive administration directly to the site of damage. These hydrogels can also serve as ideal delivery vehicles for a diverse range of bioactive agents, including growth factors, anti-inflammatory drugs, steroids, and cells. The controlled release of such biologically active molecules from hydrogel scaffolds can accelerate cartilage healing, stimulate chondrogenesis, and modulate the inflammatory microenvironment to halt osteoarthritic progression.

The present review aims to describe the methods used to design injectable hydrogels, expound upon their applications as delivery vehicles of biologically active molecules, and provide an update on recent advances in leveraging these delivery systems to foster articular cartilage regeneration.

1. Introduction

Knee osteoarthritis (OA) is the most prevalent degenerative joint disease, exerting a significant toll on adults through debilitating pain and impaired functionality. Cartilage degeneration is a characteristic of osteoarthritis but notably, trauma induced cartilage defects can expedite the onset of osteoarthritis [1]. The intrinsic properties of cartilage - sparse cell density, avascularity and absence of nerves and lymphatic tissue presents an inherent barrier for effective self-repair of articular cartilage. Consequently, cartilage defects have potential to cascade into joint deterioration, weakening its function as a load-bearing interface between the articulating femur and tibia [2].

Currently, clinical interventions for the repair of cartilage defects include microfracture (MF), chondroplasty and autologous chondrocyte implantation (ACI); however, these methods suffer from the requirement of highly invasive surgery and the formation of mechanically inferior fibrocartilage-like neotissue limiting their long-term success. The last resort for clinicians for patients with severe OA is whole joint replacement, an expensive solution which inflicts a substantial burden to the National Health Service (NHS) in the UK, costing nearly £ 2 billion annually (approximately 2% of the entire budget), therefore, underlining the urgent need for novel and more efficacious therapeutic modalities to redress this exigent clinical need [3].

In light of the inherent constrained regenerative capability of articular cartilage and the shortcomings of established clinical interventions, scientists have shifted their attention towards leveraging biomaterial scaffolds as a means to augment tissue restitution. These scaffolds fundamentally serve as architectural frameworks that facilitate tissue development, offering the potential for cell incorporation and/or the introduction of biologically active agents to supply biochemical cues to exogenous or endogenous cellular entities. A plethora of research has been carried out in the field of cartilage tissue engineering resulting in

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scaffolds that utilise a variety of strategies for scaffold mediated repair resulting from various combinations of cell sources and biologically active molecules (Fig. 1A-C) [4].

Hydrogels are an extremely promising subset of biomaterials for their intrinsic features such as their biocompatibility, high water content, minimal cytotoxicity, and biodegradability. Most scaffolds require the removal of native tissue to facilitate implantation, followed by suturing or gluing to fix the scaffolds; but these traditional practices invariably curtail their efficacy due to poor lateral integration with the indigenous cartilaginous matrix [5]. Hydrogels can be designed such that they can be injected via minimally invasive arthroscopy, circumventing the need for invasive surgical procedures and results in a precise form-fitting scaffold, fully conforming with the cartilage defect without the need for fixation [6–8]. Injectable hydrogels serve the purpose of cell and biologic delivery whilst also functioning as a domain for the regeneration of the native extracellular matrix (ECM). The exploration of these systems has witnessed a substantial surge in research since the early 2000s as depicted in Fig. 1D [9,10].

Fabrication of injectable hydrogels can be carried out using various methods:

(1) In situ gelation whereby a hydrogel solution can be injected followed by solidification via crosslinking methods in situ [11].
(2) Shear-thinning, self-healing hydrogels which can be solidified ex vivo followed by injection facilitated via shear thinning mechanism followed by in situ structural recovery (self-healing) [12].
(3) Injection of crosslinked hydrogel microparticles (MPs) with their size conforming injectability [13].

In this forthcoming review, our primary objective is to provide a comprehensive and informative overview of injectable hydrogels as a promising solution to the pervasive issue of osteoarthritis (OA). We commence by assessing the intricate structure, function, and the underlying pathological mechanisms of articular cartilage before turning our attention toward the prevailing clinical interventions currently employed by clinicians, recognizing their intrinsic limitations and

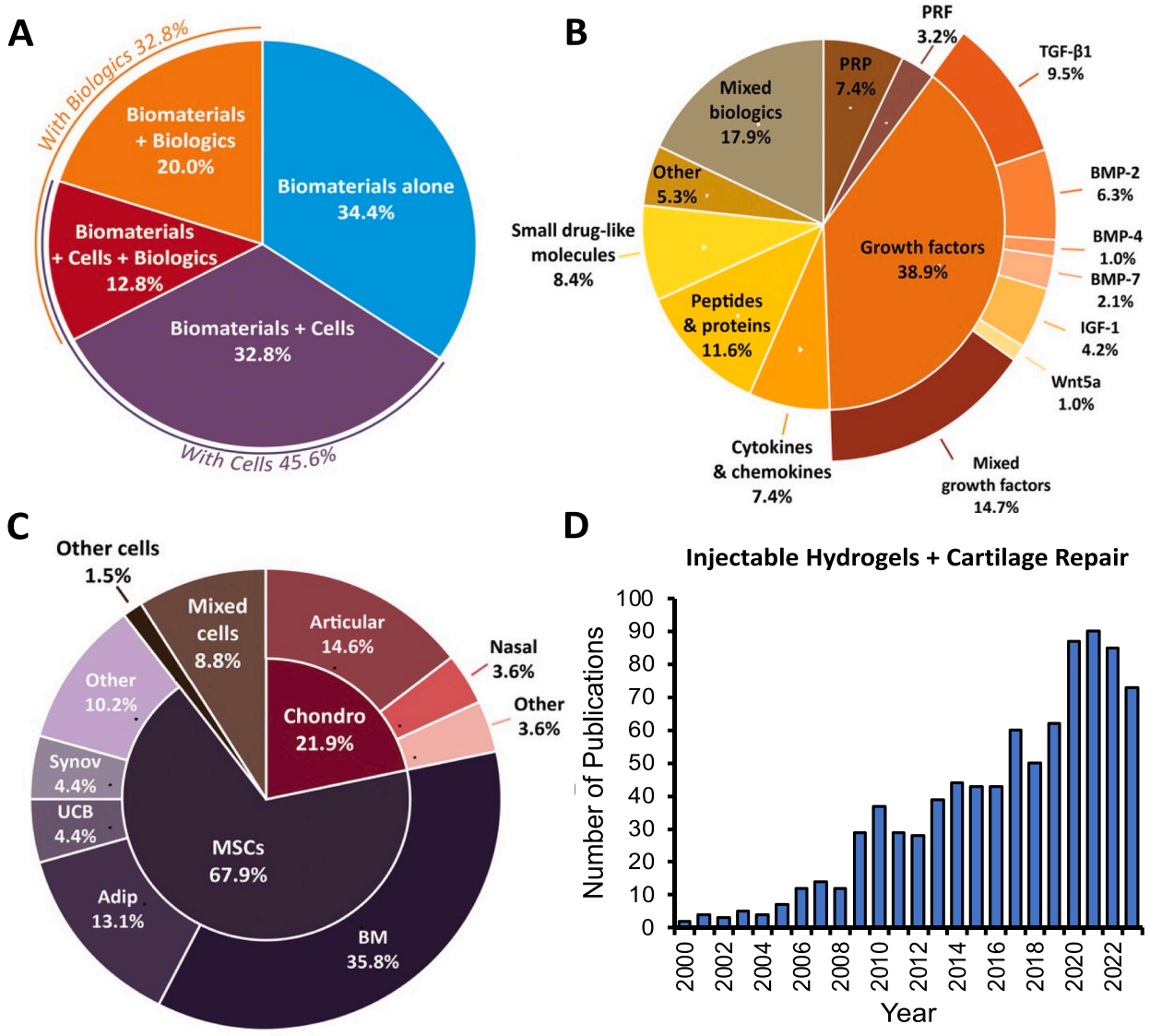

Fig. 1. Overview of recent progress in tissue engineering. A) General strategies for OC regeneration including the use of a biomaterial alone or combined with cells and/or biologics. B) Analysis of bioactive molecules used in biologics-based approaches. C) Cell types used for scaffold-based regeneration: chondrocytes (Chondro), mesenchymal stromal/stem cells (MSCs) or MSC-derived cells from the bone marrow (BM), adipose tissue (Adip), umbilical cord blood (UCB), synovial tissues (Synov) or other sources, as well as other cells or mixed cells. Reproduced from [4] under Creative Commons license. D) Number of publications listed on PubMed® using ("cartilage" and "injectable hydrogel") as the search terms displaying the increase in publications on cartilage tissue engineering approaches involving injectable hydrogels.

challenges. The focal point then shifts towards injectable hydrogels, a captivating treatment modality for the repair of chondral defects with special emphasis on the three aforementioned methods of fabricating injectable hydrogels and their very recent progress in the field of cartilage tissue engineering. Simultaneously, we describe how these hydrogels interact with the physiological environment and investigate the mechanisms governing the loading and release of biologics, shedding light on their potential as dynamic carriers of therapeutic agents, enhancing their potential for chondrogenesis. Finally, a significant portion of the discussion is dedicated to assessing commercially available and clinically relevant injectable hydrogels that are currently being used to aid the regeneration of cartilage.

2. Anatomy of articular cartilage

Within the body there are three types of cartilage tissue present: elastic cartilage, fibrocartilage, and hyaline cartilage. Articular cartilage is a hyaline cartilage found on the articulating surface of bone in synovial joints and ranges from 1-5mm in thickness. The articular cartilage is to a large extent responsible for the load-bearing and near frictionless movement of the articulating surfaces [14,15]. Unlike most tissues, articular cartilage is devoid of blood vessels, nerves, and lymphatics. It is composed of a dense ECM with a sparse distribution of highly specialized resident cells called chondrocytes [16].

2.1. ECM composition

The mechanical properties and subsequent function of the articular cartilage are dependent upon the composition of the matrix components. The ECM is made up of collagen, water proteoglycans, chondrocytes, lipids, and glycoproteins with each component providing an intrinsic function to the ECM. Water is the most abundant component of articular cartilage and contributes up to 80% of the wet weight. The high composition of water allows for the deformation of cartilage depending on the biomechanical load. Not only does water provide a medium for lubrication providing a low friction coefficient, but ions such as sodium,

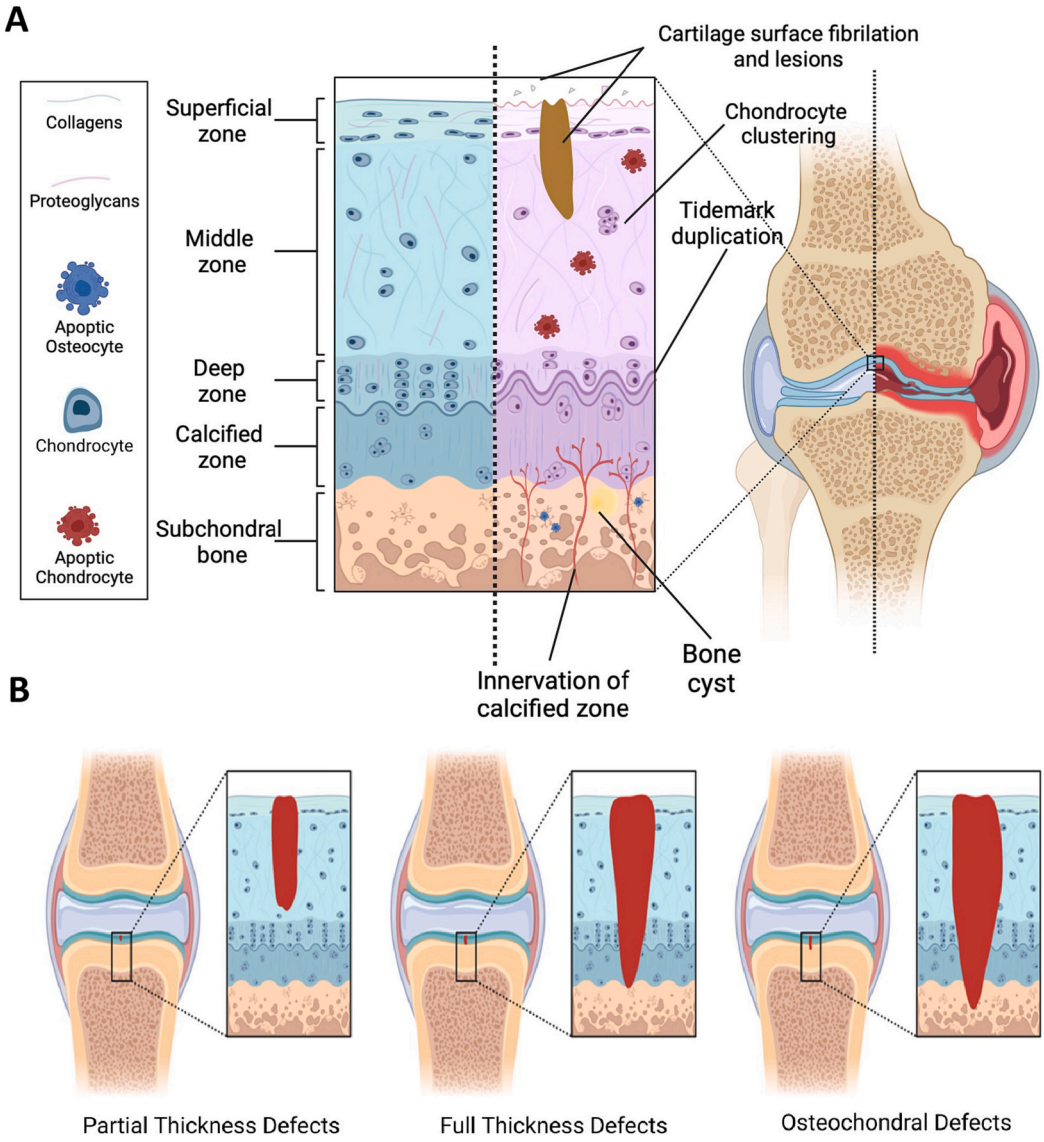

Fig. 2. Pathological changes of articular cartilage. A) Zonal composition of healthy articular cartilage vs osteoarthritic articular cartilage. The progression of osteoarthritis leads to the formation of cartilage lesions, surface fibrillations, chondrocyte clustering and hypertrophy, bone cyst formation and apoptosis of chondrocytes and osteocytes. B) Classifications of cartilage lesions because of trauma or resulting from osteoarthritis. Partial defects only penetrate a portion of the articular cartilage whereas full thickness and osteochondral defects extend through the entire cartilage with the latter extending through to the subchondral bone. (Created with BioRender.com).

calcium, chloride, and potassium are dissolved in the tissue water and the flow of water through the cartilage and across the articular surface aids the transport of nutrients to chondrocytes [16]. Collagen accounts for a major fraction of the dry weight in articular cartilage (50-80%). Type II collagen in the form of cross-linked microfibrils has been shown to form throughout the ECM. These fibrils make connections with other tissue-specific collagens of the cartilage, such as types IX and XI collagen among others (Type VI, X, XII and XIV) although the latter are sparse in abundance relative to Type II collagen [17]. These interconnected collagen fibrils possess high tensile strength, providing the ECM with the ability to withstand constant tension, even in the unloaded state due to the swelling pressure of the water/proteoglycan gel [18]. Proteoglycans are the second most abundant macromolecule in the articular cartilage ECM. A proteoglycan monomer consists of a core protein to which one or more glycosaminoglycan (GAG) chains are attached. The most prominent proteoglycan in cartilage is aggrecan whilst the most common GAGs in articular cartilage are hyaluronan, chondroitin sulphate and keratin sulphate [12]. The GAG/proteoglycan aggregates form gels which occupy a large volume relative to their mass [18].

2.2. Zonal organisation

Articular cartilage can be split into four distinct zones between the articular surface and the subchondral bone: superficial tangential zone, middle zone, deep zone and calcified zone (Fig. 2A). The zones are defined by the morphology of chondrocytes, orientation of the Type II collagen fibres and composition of proteoglycans which all differ across the zones. Chondrocytes originating from different zones also have distinct roles within cartilage.

The superficial zone is the thinnest layer of articular cartilage making up 10-20% of the total thickness [19]. Chondrocytes in this zone are high in density and are flattened. Type II collagen fibrils are oriented parallel to the articular surface providing protection to the deeper layer through its high tensile strength and resistance to shear forces during articulation. The zone is in contact with synovial fluid which aids with lubrication of the cartilage surface. The middle zone which is also known as the transitional zone contains round chondrocytes and collagen fibres are randomly orientated. The zone is also termed transitional zone as it serves as a transition between the superficial and deep zones with the collagen fibres increasing in thickness with cartilage depth [20]. The middle zone makes up 40-60% of the thickness of articular cartilage and contains the highest composition of proteoglycans compared to other zones and is the first line of resistance against compressional forces. The deep zone makes up 30% of cartilage and is characterised by columns of ellipsoidal chondrocytes distributed between thick, perpendicularly arranged collagen fibres, coupled with the highest proteoglycan concentration allows it to contribute the highest compressive resistance out of all layers. The calcified zone is distinguished from the deep zone by the tide mark. The zone is the highly mineralised region of articular cartilage and provides attachment of cartilage to the subchondral bone by anchoring collagen fibrils of the deep zone to the bone.

2.3. Function of articular cartilage

The primary function of articular cartilage is to provide a smooth, lubricated surface that facilitates the transmission of biomechanical loads with a low coefficient of friction, thereby aiding distribution of loads between opposing bones in a synovial join [15]. The biomechanical load predominantly consists of compression during basic movement in addition to shear forces during dynamic movement such as twisting, pivoting, and sliding motion. For the facilitation of this biomechanical loading articular cartilages possesses several intrinsic mechanisms for facilitation of biomechanical loading as a result of interstitial fluid flow and contribution from the collagen and proteoglycan and GAG network [16]. Keratin sulphate and chondroitin sulphate in articular cartilage carry a negative charge, creating a high affinity for water. This property helps the cartilage resist compressive loads by increasing osmotic pressure due to cation influx. During loading, the negatively charged sulphated GAGs are pushed closer together, leading to increased repulsive forces and higher compressive stiffness in cartilage. Furthermore, the flow of water through charged regions of the proteoglycan-rich matrix generates piezoelectric charges which modulates water flow and contributes to the viscoelastic nature of articular cartilage [18].

To understand the biomechanical behaviour of articular cartilage, it is best viewed as a biphasic medium, consisting of two phases: a fluid phase and a solid phase [15]. The solid phase comprises proteoglycans, collagens, and cells, while the fluid phase consists of interstitial fluid. Under impact loads, water flows through the solid permeable matrix which generates frictional drag on the matrix. The permeable nature of articular cartilage allows fluid to flow through the ECM. As a result, articular cartilage has an inherent self-protective feedback system which stiffens during loading exhibiting creep and stress-relaxation behaviour [16,21]. Overall, articular cartilage's intricate structure and interplay between its solid and fluid phases allow it to efficiently distribute biomechanical loads, resist compression, and maintain its viscoelastic properties during different types of movement.

2.4. Articular cartilage pathology

As a result of the cartilage load-bearing function, the susceptibility to and incidence of cartilage damage is high, especially during highly physical activities. Osteoarthritis arises due to chondrocytes failing to maintain homeostasis between synthesis and degradation of its ECM components. Osteoarthritis can be classified as either primary (idiopathic) or secondary. The pathophysiology of OA is still yet to be completely understood but idiopathic OA from risk factors such as age, gender and weight are known to play a part [22]. With aging, the capacity of cells to synthesize proteoglycans, proliferate and their response to anabolic stimuli such as growth factors are all factors that deteriorate [20], thus leading to the degeneration of cartilage when exposed to biomechanical loading [23].

The relationship between obesity and OA has been well defined, and studies have shown there is a strong positive correlation between body mass index and prevalence of OA[24]. Whilst the correlation between obesity and OA is partially attributed to the excessive loading on the weight bearing portion of the cartilage, obesity is in part a metabolic syndrome and is thought to contribute to systemic inflammation through secretion of pro-inflammatory cytokines interleukin (IL)-1β, IL-6, IL-8, and tumour necrosis factor alpha (TNF-α) which may trigger the nuclear factor-κB signalling pathway to stimulate an articular chondrocyte catabolic process and lead to ECM degradation via matrix metalloproteinase (MMP) upregulation [1,25,26].

There is significant evidence demonstrating that females are far more at risk of OA compared to males with 18% of women over the age of 60 affected by OA in comparison to 9.6% of men of the same age [27]. This may result from changes in hormone levels during menstruation as well as menopause [28,29], differences in the biomechanics and musculoskeletal system [30] and the increased incidence of obesity in women [31] contributing to this risk factor.

Secondary OA can derive from other diseases associated with the diarthrodial joints, congenital defects, malalignment, and injury to the joint leading to post-traumatic osteoarthritis. Typically, injuries to the joint such as bone fractures, ligament tears, meniscus damage leading to joint misalignment cause disruption to normal joint mechanics and eventual breakdown of cartilage due to uneven loading. In both primary and secondary OA, degeneration of cartilage is prevalent and either leads to the onset or progression of osteoarthritis and is accompanied by cartilage softening, fibrillation of the superficial layers, fissuring and diminished cartilage thickness, serious tissue defects that induce pain, immobility, and eventual joint destruction (Fig. 2A) [32,33].

Defects are categorised based on the depth of the lesion as: partial

thickness, full thickness, or osteochondral (Fig. 2B). Full thickness and osteochondral defects extend to the subchondral bone and can potentially be partially repaired by mesenchymal stem cells residing within the bone marrow, nonetheless self-repair is particularly limited owing to the formation of less functional fibrocartilage[34]. As a result, more rigorous treatment modalities are required and can be classified into three groups of symptomatic relief, reparative/replacement procedures, and regenerative methods.

2.4.1. *Symptomatic relief*

The end goal of symptomatic relief is to alleviate joint pain associated with osteoarthritis and provide the patient with increased mobility such that they can carry out simple everyday tasks. This is typically the first-stage strategy for clinicians when the patient is suffering from mild-severe pain (Fig. 3A).

2.4.1.1. Intra-articular injection. Whilst there is currently no food and drug association (FDA) approved disease modifying OA drug, IA injections as treatment strategies have widely been used. IA injection of non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids has been employed since the 1950s to relieve joint pain related to osteoarthritis [35]. Corticosteroid injections have been shown to decrease acute episodes of pain and increase joint mobility but at the same time, side-effects include reactive flares, gastrointestinal irritation, hypercortical

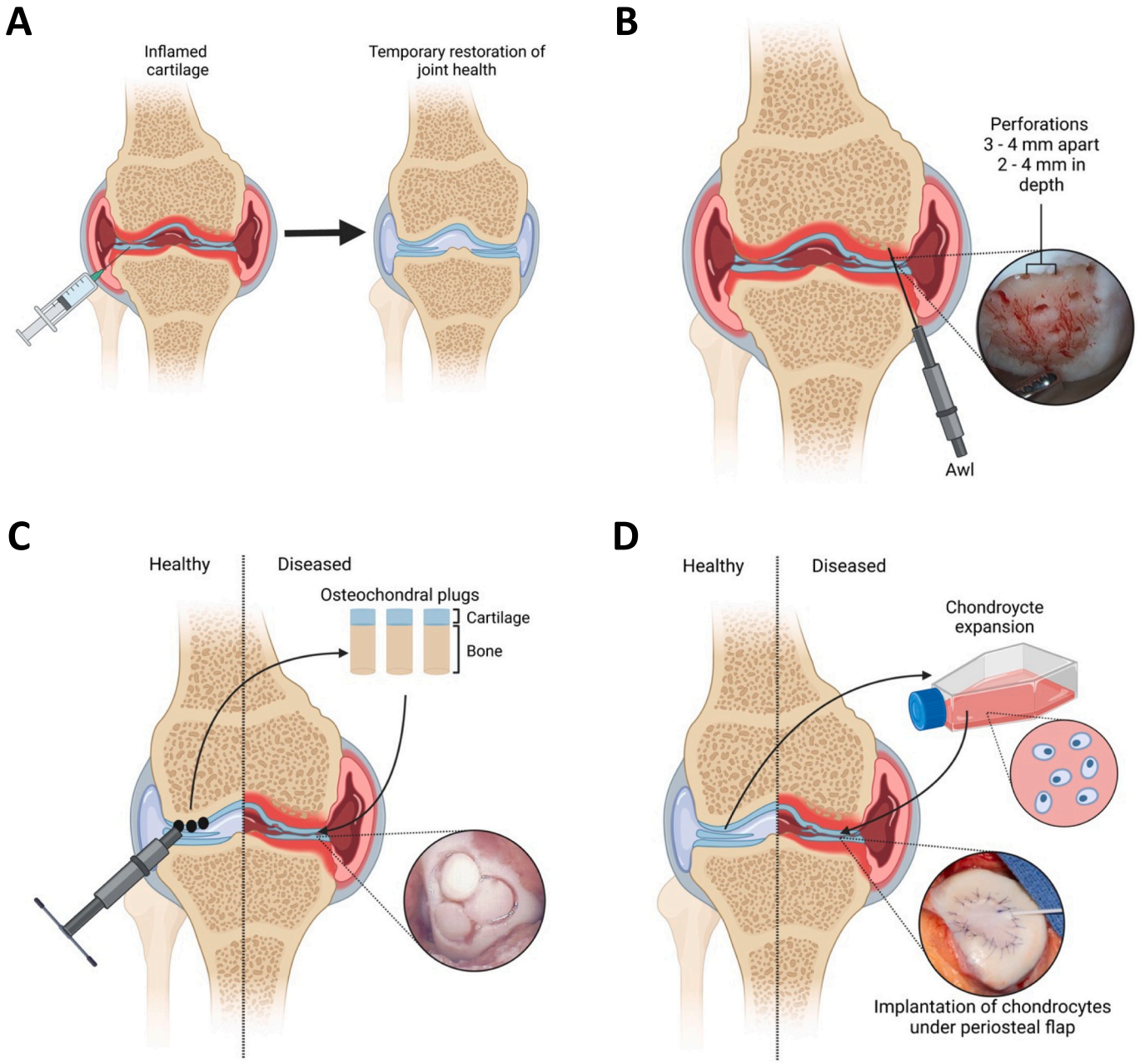

Fig. 3. Treatment methods for the repair of articular cartilage lesions. A) Early joint pain and inflammation can be suppressed by the injection of NSAIDs, steroids, PRP or viscosupplements to temporarily restore joint function to the patient. B) Bone marrow stimulation via microfracture. An awl is used to create perforations 3-4 mm apart and 2-4 mm in depth with the aim of recruiting stromal cells from the residing subchondral bone marrow. Reproduced from [61]. C) Osteochondral autograft/allograft – osteochondral plugs are taken from the healthy non-weight bearing portion of cartilage or from donors which are subsequently transplanted in a 'mosaic-like' pattern. Reproduced from [62] with permission from Elsevier. D) Autologous chondrocyte implantation – autologous chondrocytes are isolated, expanded in vitro and subsequently implanted onto a periosteal flap or collagen membrane (MACI) [63] (Created with BioRender.com).

syndrome, and osteoporosis and there are also conflicting reports as to whether IA injection of corticosteroids can exacerbate joint destruction [36]. Furthermore, corticosteroids are only applicable for symptomatic pain and rarely effective for the treatment of inflammation [37].

Synovial fluid (SF) is a critical component to the function of healthy joints with approximately 2ml of SF in each knee joint. Hyaluronic acid (HA) is major component of SF with a concentration of 2.5-4.0 mg/mL and a molecular weight of 4-10 MDa which provides SF with viscoelastic properties essential for shock-absorbance and lubrication [38,39]. However, during OA both the concentration of HA and the molecular weight decreases, causing the reduction of SF viscoelasticity and its subsequent ability to protect cartilage [40]. The concept of viscosupplementation was developed by Balazs in the 1990s who hypothesized that the injection of exogenous HA could restore the viscoelastic properties of SF to the osteoarthritic knee joint [41]. Viscosupplementation has been recommended by several societies of rheumatology, orthopaedics and sports medicine and has since been used for >20 years with many commercially available viscosupplements, each with variations in polymer composition and molecular weight [42].

Platelet-rich plasma (PRP) is defined as an autologous blood product which contains an elevated concentration of platelets above that of whole blood. Preparation techniques vary but typically, one-step or two step centrifugation is carried out to separate red blood cells and the supernatant is composed of plasma with a high concentration of platelets [43]. The resulting PRP contains a plethora of proteins, growth factors and anti-inflammatory cytokines and is injected into the knee joint to induce cellular proliferation, migration, and differentiation, making it a suitable therapeutic agent for cartilage tissue engineering [44,45]. However, the use of PRP is not recommended by several institutions owing to the lack of standardisation with the preparation protocol [46,47].

2.4.2. Reparative procedures

Naturally, cartilage has very limited self-repair ability and recruits cells from the synovial membrane or from the subchondral bone depending on the extent of the trauma. In any case, the original state of the cartilage is never recovered, instead mechanically inferior fibrocartilage is formed [48].

2.4.2.1. Bone marrow stimulation techniques. The first bone marrow stimulation procedure was carried out by Pridie in 1959 where a drill was used to form subchondral perforations with the aim of releasing mesenchymal stem cells and growth factors to enhance the production of cartilaginous tissue [49,50]. However, the use of a motorised drill led to concerns regarding osteocyte thermal necrosis. To overcome this, Steadman carried out a procedure termed microfracture whereby perforations 3-4 mm apart and 2-4 mm in depth were created using an awl instead of a drill to alleviate thermal necrosis (Fig. 3B). The procedure is typically used for small lesions (>2 cm^2) and has been shown to reduce pain and increase mobility for patients, however, the procedure has limited long-term success [51], likely due to the production of mechanically inferior fibrocartilage composed of type I collagen rather than Type II collagen that is native to articular cartilage. The weaker fibrocartilage consequently degrades after long term-exposure to biomechanical loading, typically 18-24 months post-surgery [52,53].

2.4.2.2. Osteochondral autograft (mosaicplasty) and allograft. The transplantation of donor tissue typically in the form of cylindrical plugs from the non-weight bearing portion of cartilage from either the patient (autograft also known as mosaicplasty [54]), or from another individual's cartilage (allograft [55,56]) to replace defected cartilage has been carried out since 1970s (Fig. 3C). Whilst the procedure can fill small and even large defects effectively with native hyaline cartilage, it is limited by donor site morbidity and lack of lateral integration with the native cartilage eventually leading to cyst formation [57].

2.4.3. Regenerative approaches

2.4.3.1. Autologous chondrocyte implantation. Inability of MF to successfully treat large lesions led to the development of autologous chondrocyte implantation (ACI) [58]. ACI is a form of cell therapy, more commonly used to treat larger lesions (3-10 cm2). Essentially, it is a three-stage process which first entails harvesting a cartilage biopsy from the non-weight bearing portion of the patient's articular cartilage, isolating the chondrocytes by means of enzymatic digestion of the surrounding matrix, followed by expansion of the chondrocyte numbers by culturing in-vitro. The last stage is the implantation of the chondrocytes under open-knee surgery with a periosteal flap (Fig. 3D). Comparative studies of ACI with MF have shown MF is effective in repair of small lesions but ACI is more effective in treating larger lesions and produces a higher proportion of hyaline cartilage [59]. The use of ACI is associated with its own disadvantages including donor-site morbidity, limited availability, and de-differentiation during mono-layer expansion [60]. Later iterations of ACI saw the use of artificial matrices such as porcine collagen membranes or hyaluronic acid scaffolds instead of a periosteal flap with these procedures termed as matrix-assisted autologous chondrocyte implantation (MACI). Whilst MACI alleviated graft issues with the periosteal flap, the major limitation of any ACI procedure is the requirement for multiple operative procedures. Nevertheless, the reasonable success of ACI and MACI effectively pioneered tissue engineering and the development of scaffolds for tissue engineering.

2.4.3.2. Cell therapy. First identified in 1966, cells with potential to differentiate into any lineage are known as stem cells. There are two main classifications of stem cells: adult mesenchymal stromal cells (MSCs) and embryonic stem cells (ESCs).

There is controversy around the labelling of MSCs as stem cells because they were first believed to have multi-lineage differentiation capacity and the ability to differentiate into tissue-forming cells, however we now understand that their therapeutic effect arises from secretion of bioactive factors such as extracellular vesicles [64,65]. Regardless of the controversy, MSCs have been used extensively as a cell source for cartilage regeneration [66]. Their high self-renewal ability and ability for multipotent differentiation to various relevant primary cells such as adipocytes, osteoblasts and chondrocytes make them an attractive cell source for tissue engineering applications [67,68]. Originally identified in bone marrow, MSCs or MSC-like cells are easily obtained from many sources such as umbilical cord, adipose tissue, and synovium, and have been shown to differentiate to chondrocytes even after expansion [69]. Studies comparing the use of ACI with primary chondrocytes and MSCs (implanted with the same matrix) showed both groups significantly improved the patients' quality of life with no significant difference between the two groups. However, since MSCs can be obtained in a minimally invasive manner in contrast to the isolation of chondrocytes, added to the fact that MSCs proliferate faster than chondrocytes, the use MSCs is far more time and cost-effective [70,71].

Chondroprogenitors are a population of cells found in various tissue and possess primed for chondrogenic potential. They are isolated from cartilage biopsies enzymatically but the chondroprogenitor population are separated and sorted from chondrocytes as a result of their MSC surface markers CD105, CD9, CD90, CD166, and CD146 as well their ability to adhere to fibronectin [72,73]. Compared to chondrocytes which lose their potential to differentiate after several passages in culture, chondroprogenitors can be expanded in culture for many generations whilst retaining the ability to undergo chondrogenic differentiation. In vivo animal studies have shown chondroprogenitors show more promise at repairing cartilage defects as compared to chondrocytes and MSCs [74–76]. There is currently a phase II clinical trial utilising allogenic chondroprogenitors as a therapy which could prove promising if successful [77].

ESCs are pluripotent with unlimited self-renewal, which ultimately

makes them a promising cell source for cartilage regeneration. Studies have shown that ESCs undergo chondrogenic differentiation when activated by growth factors, can alleviate osteoarthritis through modulation of homeostasis and form hyaline cartilage [78–80]. However, a significant drawback of ESCs which has limited their clinical application is their origin and the death of an embryo during isolation of ESCs which has consequently raised ethical issues with research utilising these cells in relation to the sanctity of life.

iPSCs are somatic cells that are reprogrammed by delivery of transcription factors to induce pluripotency. iPSCs share similar properties to ESCs such that they exhibit similar surface markers, morphological characteristics, and gene expression yet iPSCs do not have the same ethical concerns related to them as ESCs do [81]. iPSCs are a promising strategy in regenerative medicine; however, their novelty means there is a lack of understanding of their chondrogenic differentiation as well as poor standardisation of protocol for chondrogenesis [82].

3. Cartilage tissue engineering

The promising nature of regenerative procedures such as ACI, as well as the advantages of using a scaffold to support cell-growth as shown in MACI have demonstrated promise. However, the burden of multiple surgical procedures and potential donor site morbidity, when contrasted with the relative success of MSC injections has led to researchers in the field of tissue engineering to focus on the development of injectable scaffolds to not only aid the regeneration of cartilage but also as delivery vehicles of cells as well as other therapeutics [6,8,83,84]. As a result, a comprehensive summary of recent advances in this field are discussed.

The fundamental principles of tissue engineering scaffolds are to: (1) to provide a porous supporting structure to enhance proliferation of cells in the underlying cartilage or bone form new tissue, (2) to possess mechanical properties to withstand biomechanical loading applied to the scaffold in vivo, (3) to be biocompatible with local tissue as to reduce host response but maximise cell growth and tissue integration, (4) to be biodegradable such that the scaffold can initially support cell growth but degrade to allow for tissue growth and optionally (5) to act as a delivery vehicle and provide protection for cells, biologically active molecules or drugs that are loaded in vitro [85,86].

Materials such as bioceramics and metals have been employed as biomaterials for scaffolds, however these materials are far too stiff to be utilised in cartilage tissue engineering and are much more commonly applied in bone tissue engineering [87]. Removing cells and genetic material from the native ECM to form a scaffold composed of the natively complex microenvironment, histoarchitecture, with optimal composition of proteoglycans, GAGs and collagen which are all components that are conducive to chondrogenic differentiation and proliferation is an attractive prospect that has shown success in cartilage tissue engineering [88]. However, issues with standardisation and poor reproducibility of these scaffolds limits the clinical application of decellularized ECMs.

For the past decade, polymers in the form of hydrogels have widely been considered the material of choice for soft tissue engineering applications. Hydrogels are a three-dimensional network of water-soluble polymers which are chemically or physically crosslinked [89]. Their hydrophilicity originates due to the presence of hydrophilic moieties such as carboxyl, amide, amino and hydroxyl groups which exist along

Table 1
Natural and synthetic hydrogels for cartilage tissue engineering with their respective advantages and disadvantages.

Classification	Material	Advantages	Disadvantages	Ref.
Natural	Alginate	• Natural polysaccharide • Fast gelation • Good viscoelastic properties • Biocompatible • Highly abundant and inexpensive • Native component of connective tissue	• Poor mechanical strength • Poor adhesive properties • Lack of anchoring sites for cells	[101–103]
	Chitosan	• Complexation • Bacteriostatic • Absorbability • Anti-oxidation • Promotes cell proliferation • Good adhesion due to positive charge	• Poor mechanical strength • Low solubility • Relatively expensive • Short retention time • Expensive to purify	[104–106]
	Gelatin	• Thermally reversible with transition close to physiological temperature • Promotes cell adhesion due to possession of RGD sequence • Relatively high mechanical strength • Low cost	• Long gelation time • Viscous • Unstable at high temperatures	[107–109]
	Hyaluronic Acid	• Natural component of synovial fluid and ECM to mimic microenvironment • Good viscoelastic properties • Promotes cell proliferation • Lubrication properties, water solubility, low immunogenicity	• Poor mechanical strength • Relatively expensive • Short retention time	[110–112]
	Chondroitin Sulphate	• Native component of cartilage tissue • Stimulates chondrocytes to produce ECM. • Water solubility • Inexpensive	• Poor mechanical strength • Fast degradability	[113–115]
	Collagen	• Most abundant protein found in cartilage. • Excellent biocompatibility and biodegradability	• Limited groups for functionalisation • Low mechanical strength • Potential for host immune response	[116,117]
Synthetic	Silk Fibroin	• High mechanical strength • Similar in structure to collagen	• Low biodegradability	[118]
	PEG	• Biocompatible • Easily functionalised and combined with other polymers	• Poor cellular interaction	[119,120]
	PVA	• Good viscoelasticity • Biocompatible	• Non-degradable • Poor cellular interaction	[121,122]
	PAAm	• Biocompatible	• Poor mechanical strength • Poor cellular interaction • Low biodegradability	[123]
	PNIPAM	• Thermosensitive with gelation occurring close to physiological temperature.	• Poor mechanical strength • Low drug loading capacity	[124,125]

the backbone of polymeric chains endowing hydrogels with the ability to absorb high amounts of water (typically 70 - 99%), thus making them physically similar to native articular cartilage [90].

The physiochemical properties of hydrogels are highly dependent on the base polymer(s) that are used to create the hydrogel network. These polymers can be classified into two main groups, natural and synthetic (Table 1). Natural polymers can be further sub-categorised into protein based (collagen, elastin, fibrin, gelatin, silk fibroin) and polysaccharide based (GAGs, alginate, and chitosan). Hydrogels composed from natural polymers frequently have many benefits such as excellent biocompatibility with minimal immune response, they also possess great biochemical signalling and the ability to mimic the native articular cartilage ECM with cell-controlled degradability [91–93]. However, naturally derived hydrogels suffer from poor mechanical strength, high rates of degradation, as well as being expensive to source and process. To overcome their poor mechanical properties, natural polymers can be chemically modified owing to the existence of various chemical groups (hydroxyl, carboxyl, amine, thiol) which are vastly abundant in natural polymers and can be conjugated to form esters, amides, ethers and carbamates and can thereafter form crosslinks with many other polymers[94,95].

Hydrogels that derive from synthetic polymers such as poly(ethylene glycol) (PEG), Poly(acrylamide) PAAm, and poly(N-isopropyl acrylamide) PNIPAM often possess great mechanical properties that can be tuned by altering processing parameters. Although PEG, PAAm and PVA are approved by the FDA, cytotoxicity of these synthetic biomaterials must be monitored due to use of potentially toxic reactants in their synthesis, negatively impacting their biocompatibility [96]. In contrast to natural hydrogels, synthetic hydrogels possess poor biological properties owing to the lack of cell-matrix interactions such as cell-adhesion and cell-mediated biodegradation. In order to overcome this limitation, bioactive molecules can be incorporated into synthetic hydrogels; cell-adhesive peptides [97], natural polymers [98,99] and growth factors can be incorporated as we will later detail [100].

4. Fabrication of injectable hydrogels

Whilst hydrogels in general are arguably the most promising types of scaffolds currently being developed, injectable hydrogels can offer the additional benefit of minimally invasive implementation via intra-articular injection, in contrast to preformed scaffolds. The number of arthroplasties required to be performed in the US annually by 2030 is projected to be a staggering ~3.5 million [126–128], as such, injectable hydrogels have the potential to drastically reduce this number. The 3D network of injectable hydrogels allows for the encapsulation of cargo such as cells, drugs and other therapeutics which can augment traditional therapies such as injection of corticosteroids, ACI/MACI and MSC therapy [129,130]. Furthermore, they can fill any shape or defect unlike pre-formed scaffolds which must be designed to a specific shape or require the removal of healthy tissue to facilitate the implantation of the scaffold. Injectable hydrogels can be broadly classified as: in situ gelling, shear-thinning hydrogels, and micro-/nano- hydrogel systems.

4.1. *In situ gelling hydrogels*

In situ gelling hydrogels can be described as systems that are injected as a polymer solution (typically referred to as a precursor solution), once the solution has been injected to the defect site, the hydrogel is formed after the characteristic sol-gel transition due to either physical or chemical crosslinking (Fig. 4).

4.1.1. *Michael addition*

Michael addition reactions are the nucleophilic addition of a nucleophile to an unsaturated carbonyl compound (Fig. 5A) [131]. Thiol-ene Michael additions are one of the most common reactions for the formation of hydrogels taking place between polymers functionalised with thiol moieties and polymers with double bonds such as vinyl sulfones, maleimides, norbornenes, acrylates and allyl ethers. Several studies have designed promising hydrogels based on PEG and HA by thiol-Michael addition for cartilage tissue engineering purposes, with Li et al. using a PEG-HA hydrogel prepared by thiol-ene reaction as an injectable scaffold for chondroprogenitors exhibiting acceleration of ECM production and the ability to retain the phenotype and function of encapsulated chondroprogenitors [132–134]. A severe limitation of this mechanism is that the gelation time is relatively slow. Although tuning of the gelation time can be carried by changing precursor pH and concentration, Liu et al. reported an *in-situ* gelation time of 30 minutes [134], as a result the polymer solution has time to disperse from the defect site thus limiting the clinical application these systems.

4.1.2. *Thermosensitive*

Temperature responsive in situ gelling hydrogels have been extensively researched in tissue engineering due to their ability to rapidly gel because of change from room temperature to physiological temperature (37°C) inside the cartilage defect (Fig. 5B). The mechanism of these hydrogels is because of interactions between hydrophilic and

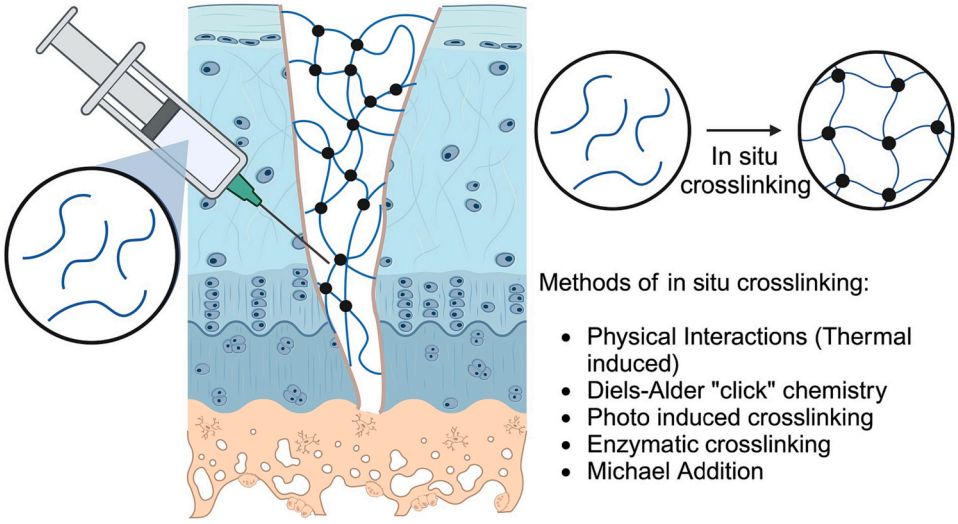

Fig. 4. Schematic overview of in situ gelling mechanisms. The hydrogel pre-polymer is injected as a solution followed by gelation in situ as a result of one of the various crosslinking mechanisms discussed in this chapter (Created with Biorender.com).

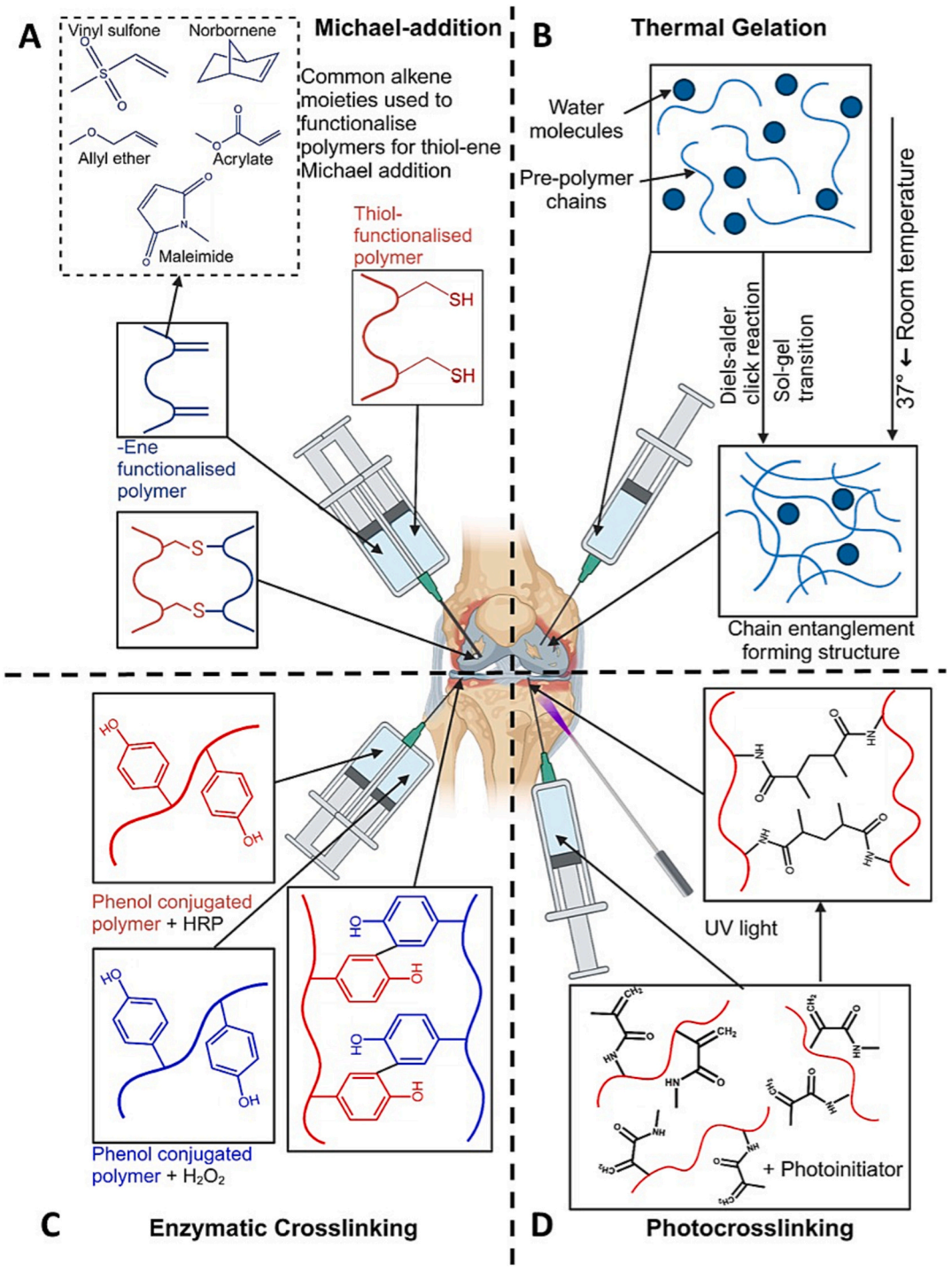

Fig. 5. In situ gelling hydrogel systems. A) Schematic of thiol-ene Michael addition reaction between -thiol and -ene functionalised polymers. These systems are typically employed as a dual-barrel syringe, after extrusion the thiol moieties take part in a nucleophilic addition with the alkene modified polymer to from a crosslinked hydrogel. B Schematic of thermal gelling hydrogel. A pre-polymer solution is injected into the defect where the thermal transition occurs whether it is chain entanglement, Diels-Alder reaction or intermolecular physical interactions to form a structured hydrogel [133]. C) Schematic of enzymatic crosslinking mediated by HRP and H_2O_2. Phenolic conjugated polymers act as reducing agents in the presence of H_2O_2 and HRP. The reaction involves the transfer of electrons from the phenolic groups to the hydrogen peroxide, resulting in the oxidation of the phenol groups and the formation of radical species. The formed radical species react with neighbouring polymer chains and formation of covalent cross-links between polymer chains within the hydrogel. D) Schematic of photocrosslinking. Polymers functionalised with acrylate/methacrylate groups with the latter more common are mixed in solution with a photoinitiator typically Irgacure 2959 or LAP. The solution can be injected into the defect in vivo and exposed to a particular wavelength of UV light. Excitation of the photoinitiator leads to the formation of free radicals which attack the double-bond on the acrylate leading to chain reaction and forming a stable covalently crosslinked structure (Created with BioRender.com).

hydrophobic domains. Temperature increase yields the dehydration of polymer chains leading to the formation of hydrophobic domains and eventually transition of an aqueous liquid to a hydrogel network because of increase in entropy. Due to the absences of any potentially harmful chemical crosslinkers, thermosensitive hydrogels are extremely promising for tissue engineering purposes.

Collagen hydrogels are arguably the most common thermoresponsive hydrogels for cartilage tissue engineering with collagen derived scaffolds being one of the early matrixes used in the very first MACI procedures [135]. Whilst the native cartilage is highly abundant in type II collagen, pure type II collagen hydrogels tend to be very weak. The most frequently used collagen scaffolds tend to be composed from type I and type III which are more mechanically robust than type II collagen. Recently, Kilmer et al. utilised a blend of type I and II collagen hydrogels which not only overcame limitations associated with mechanical stability but promoted chondrogenic differentiation of MSCs and recruited native chondrocytes to form repair tissue using an in vivo rabbit model [136]. In addition to their excellent biocompatibility and biodegradability, their ability to form a gel at physiological temperature makes them a suitable candidate for minimally invasive implementation [137–139]. Gelatin is a denatured form of collagen but retains the molecular characteristics present in collagen which are vital for cell adhesion and signal transduction. These characteristics play a significant role in maintaining the chondrocyte phenotype. In comparison to collagen, at lower temperatures (~25 °C) gelatin molecules self-assemble to form triple helix structures therefore by itself gelatin would be inadequate to use a hydrogel for tissue engineering, therefore chemical modifications to functionalise gelatin for photocrosslinking or enzymatic crosslinking are necessary [140–142].

Chitosan (CS) is a naturally derived cationic polysaccharide possessing great biocompatibility and biodegradability and its positive charge gives it great adhesion to the negatively charged native proteoglycans and is thus an attracting candidate for use in tissue-engineering applications. Chitosan can be mixed with β-glycerophosphate (GP), as an ionic crosslinking agent, to create an in situ thermal-sensitive gelling system, whereby increasing GP concentration leads to a decrease in lower critical solution temperature (LCST) since GP modulates intermolecular forces involved in gel formation as a result, gelation temperature can be finely tuned [143]. The chitosan/GP system has been shown to support cell survival and proliferation of MSCs and differentiation towards cartilage-like tissue with its positive charge also allowing for electrostatic interaction with the negatively charged cartilage [144–146].

One of the most utilised thermo-responsive synthetic polymers is PNIPAM which can solidify in situ without the addition of cytotoxic crosslinkers, initiators, or catalyst molecules. Limitations of PNIPAM is its high level of syneresis resulting in a low equilibrium swelling ratio, instability at physiologic temperatures [147]. However, since the sol-gel transition occurs at temperatures around 32 °C which is close to physiological temperature (37 °C), studies have shown that the temperature of the joint may decrease beyond this, thus affecting its suitability as a polymer for cartilage repair [148]. As a result, PNIPAM is often modified with natural polymers to alleviate its poor mechanical properties and limited biocompatibility. For example, HA [149], gelatin [150] and CS [151] have all been integrated with PNIPAM to form injectable thermosensitive hydrogels with promising outcomes. Whilst PNIPAM is a promising biomaterial for cartilage tissue engineering, at the time of this review, a major limitation of PNIPAM is that it is still not FDA approved.

Alternatively, Pluronic® F-127 is a triblock PEO–PPO–PEO copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO). The PEO block is hydrophilic and water soluble while the PPO block is hydrophobic and water insoluble. In an aqueous environment, these block copolymers self-assemble into micelles with a hydrophobic PPO centre core and a hydrophilic PEO outer shell that interfaces with water. Due to their amphiphilic nature, lipophilic molecules can be stored inside the hydrophobic core making Pluronic micelles effective thermosensitive drug transporters [152]. The sol-gel transition and resulting structural properties can be tuned by varying the concentration of Pluronic [153]. Pluronic F-127/ Poloxamer 407 is commonly used in biomedical applications due to its non-toxicity, biocompatibility, biodegradability, ability to support cell attachment and collagen formation. Garcia-Couce et al. recently developed an injectable hydrogel composed of PF-127 grafted with the aforementioned chitosan to develop a hydrogel for dexamethasone delivery. Whilst the addition of chitosan led to decreased gelation time (~100s) at 37 °C, there was a more sustained release of dexamethasone upon coupling with PF-127 [154]. The same combination of PF-127 and chitosan was employed as an injectable chondrocyte delivery vehicle which exhibited chondrocyte proliferation and ECM expression after 28 days [155].

Whilst the thermos-sensitivity of these hydrogels aid with injectability at room temperature followed by gelation at the defect at physiological temperature, the major limitation with thermosensitive hydrogels formed via physical interactions is that the nature of the physical bond is that they are inherently weak. Given that the defect is typically located in weight bearing portion of the cartilage, a semblance of mechanical strength is necessary for long term implant structural rigidity.

To overcome the limitations of poor mechanical properties of physically crosslinked thermos-responsive hydrogels, the Diels-Alder (DA) reaction can be used to prepare hydrogel via stronger covalent bonds. The DA reaction is a thermoreversible "click" reaction between orthogonal molecules typically a diene and a dinucleophile with the reaction proceeding at 20-80 °C and is free from side reactions and by-products. Most commonly, polymers functionalised with furan and maleimide groups are typically used as the diene and dienophile respectively. Despite the reaction being accelerated in water, the DA reaction between furan and mal can take hours to days to proceed at physiological temperatures, as a result, many researchers utilise DA reaction as a secondary crosslinking method in situ whilst employing a faster, more stable primary crosslinking method first [156]. Alternatively, fulvenes can be utilised instead of furan as the diene which exhibit 10 times faster crosslinking time compared to furan-mal hydrogels [157].

4.1.3. *Enzymatic crosslinking*

Enzymatic crosslinking is a form of chemical crosslinking whereby the chemical reaction is mediated by the presence of enzymes. Several injectable hydrogels formed via enzymatic crosslinking for cartilage tissue repair have been reported utilising a variety of enzymes as catalysts such as tyrosinase, transglutaminase, phosphates, thermolysin, oxidases and peroxidases [158,159]. The benefit of these crosslinking systems lies within the reaction taking place under mild conditions and does not generate toxic side products as compared to other chemical crosslinking reactions. However, these natural enzymes are costly, reportedly unstable and prone to deactivation when in solution [160]. Horse radish peroxidase (HRP) mediated systems are particularly advantageous and very commonly used as they can be used in conjunction with hydrogen peroxide (H_2O_2) to crosslink phenol-conjugated polymers (Fig. 5C). This chemical crosslinking hydrogel system not only forms hydrogels under physiological conditions in a short period of time but also has excellent biocompatibility [161]. Whilst the mechanisms of release will be detailed further in this article, HRP systems are especially advantageous in drug delivery systems since crosslinking density, mechanical properties, gelation, and degradation kinetics can be tuned independently by variation of HRP and H_2O_2 concentrations [162]. These systems are typically administered with a double-barrelled syringe whereby the HRP and H_2O_2 reside in each respective chamber along with the polymer(s) being crosslinked. Upon injection, the two solutions mix and form a hydrogel post-administration. One such system is exhibited in (Fig. 5B) whereby a collagen type I-tyramine and hyaluronic acid-tyramine was fabricated as a bone marrow derived MSC-laden hydrogel system for cartilage regeneration displaying good in

vivo results [159]. Many researchers utilise tyramine due to its ease of functionalising via amidation to polymers such as collagen, dextran, PEG and commonly HA all of which have seen promising results for cartilage repair [161,163–169]. However, a major concern is the cytotoxicity and possible adverse immunological response to presence of H_2O_2 catalysing the crosslinking process with many studies reporting increasing concentration and exposure time of a variety of cells lead to increased cytotoxicity [170,171]. Due to this, injectable enzymatically crosslinked hydrogels utilizing HRP and H_2O_2 need to optimise the reaction concentrations as to achieve a fast-crosslinking time but to avoid cytotoxicity.

4.1.4. Photopolymerization

Hydrogels can be prepared through photo-crosslinking, giving them the unique ability to crosslink in response to light exposure. The precursor solution typically consists of polymer(s), therapeutic molecules, cells, and photoinitiator. Once injected into the cartilage defect, this system offers precise spatial and temporal control over gelation via exposure to UV light which can also be administered in a minimally invasive arthroscopic manner. The photoinitiator plays a crucial role in these systems, as it generates free radicals upon exposure to a specific range of wavelengths, thus initiating polymerization (Fig. 5D). Some common photoinitiators include 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959) and Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), however, at high concentrations, these can be cytotoxic and only emit free radicals when excited by UV light which is not only detrimental to cells encapsulated inside the hydrogels but also to native cells residing near the defect [172]. To overcome this, some systems are able to make use of hydrogels with photoinitiator that are excited by less detrimental visible light such as eosin-Y and ruthenium/sodium persulphate [173,174]. Many natural and synthetic polymers contain hydroxy and reactive amine groups which can undergo substitution with the acrylate/methacrylate groups rendering the polymer suitable for photocrosslinking. There is a plethora of polymers that have been functionalised for photocrosslinking for cartilage tissue engineering prospects such as PEG, collagen, gelatin, hyaluronic, silk fibroin and sericin just to name a few [175–179]. Wang et al. designed an injectable hydrogel HA-Furan and Maleimide-PEG which was first rapidly crosslinked in 30s by photopolymerization in situ, but then thermal-induced DA click chemistry further occurred at 37°C between furan groups and maleimide groups and the slow reaction gradually increased the mechanical properties of hydrogel with the hydrogel showing good viability with a chondrocyte cell line [156].

4.2. Shear-thinning and self-healing

Unlike *in situ* hydrogels, a distinct class of pre-formed hydrogels can be injected and exhibit viscous flow under shear stress (shear-thinning) which can then subsequently recover post-injection when the applied shear-stress is removed (self-healing). As a result, shear-thinning, self-healing systems do not require the precise tuning of gelation time unlike *in situ* forming hydrogels which have the potential for premature gelation and blockage of the syringe or dispersion of the precursor from the defect site prior to gelation. These hydrogels are formed from physical crosslinks (hydrogen bonds, guest-host and electrostatic interactions) as well as from dynamic covalent bonds (Schiff-base, oxime chemistry and disulfide bonds) [180].

4.2.1. Physical crosslinking

Supramolecular chemistry is based on the noncovalent binding of molecular motifs through hydrogen bonding, host-guest interactions, π-π interactions, Van-der-Waals forces, metal chelation and hydrophobic interaction [181]. These reversible non-covalent interactions allow for the recapitulation of the dynamic and viscoelastic behaviour of the native ECM and are therefore interesting candidates for hydrogels in cartilage tissue engineering.

4.2.1.1. Hydrogen bonding.

Hydrogen bonding entails an interaction between hydrogen atoms and electronegative atoms (Nitrogen, Oxygen, Fluorine). Whilst hydrogen bonds are comparably weaker than covalent bonds, the formation of multiple hydrogen bonds contributes to improved bonding strength and gelation of polymer chains [182,183]. As such, Ureido-pyrimidione (UPy) is an important motif allowing the formation of four hydrogen bonds per unit and can be incorporated via immobilisation or functionalisation to polymer chains. Owing to the reversible nature of UPy dimerization, hydrogels possess excellent self-healing properties [184]. Hou et al. grafted UPy to dextran owing to the abundance hydroxyl groups present on the dextran backbone. The hydrogel effectively formed cartilage and bone constructs which had excellent integration with one another (Fig. 6A) [185]. Another study utilised dual self-healing mechanisms, Schiff-base and UPy dimerization to form an oxidised alginate, UPy functionalised gelatin hydrogel with excellent self-healing time (2 mins). To overcome the limitation of poor mechanical strength, poly(ethylene glycol)-poly(urethane)/cloisite nanohybrid (PEG-PU/C) was incorporated into the hydrogel resulting in a 20-fold increase in compressive strength as well as positive effects on cell proliferation (Fig. 6B) [186].

4.2.1.2. Host-guest.

Hydrogels formed via host-guest are formed by the interaction between host molecules and guest molecules in a type of "lock and key" manner. Most host-guest interactions are typically mediated by cyclodextrin which has a lipophilic inner cavity and a hydrophilic outer surface but also fewer common molecules like cucurbiturils, calixarenes and crown ethers are also used. Guest molecules can fit into the binding site of the host whilst also possessing functional groups than can interact with the host molecule. As a result of the reversibility of this mechanism, hydrogels exhibit shear-thinning and self-healing properties to facilitate IA injection [187]. Jeong et al. was able to demonstrate this system whereby β-cyclodextrin modified hyaluronic acid as the host, and adamantane-modified hyaluronic was utilised as the guest to form an injectable supramolecular hydrogel exhibiting shear-thinning and self-healing properties with the hydrogel system exhibiting cytocompatibility and capability to induce chondrogenic differentiation in a rat model [188]. Since the host-guest interaction lacks mehanical stability, Li et al. designed a self-healing hydrogel composed of β-cyclodextrin and methacryloyl modified PLGA as the host and chitosan modified with methacryloyl moieties and cholic acid as the guest polymer. There was a host-guest interaction between the β-cyclodextrin and cholic acid respectively, where after injection, photocrosslinking was carried out to covalently crosslink the two methacrylate modified polymers. Adipose derived MSCs were encapsulated within the hydrogel which demonstrated excellent regeneration in a rat defect model (Fig. 7A) [189].

4.2.2. Dynamic covalent bonds

4.2.2.1. Schiff-base reactions.

Schiff-base reactions occur via nucleophilic addition of an amine group to aldehyde/ketone groups to form dynamic covalent imine bonds. They have been used extensively in the formation of hydrogels for cartilage tissue engineering owing to the crosslinking occurring at physiological conditions and non-toxic products thereafter [190,191]. Amine and aldehyde groups are vastly abundant in natural and synthetic polymers used to formulate hydrogels for cartilage tissue engineering. For example, several injectable hydrogel systems have used chitosan for Schiff-base reactions owing to the abundance of amino groups it possesses including with PEG, fibrin, dextran, HA, and chondroitin sulphate. HA and dextran are both commonly used in Schiff-base reactions since they can be chemically cleaved with sodium periodate to introduce aldehyde groups, not only does this allow for Schiff-base formation of hydrogels, but the aldehyde groups provide adhesion with the native articular cartilage tissue and allow for the delivery of amine-rich therapeutics such as platelet rich

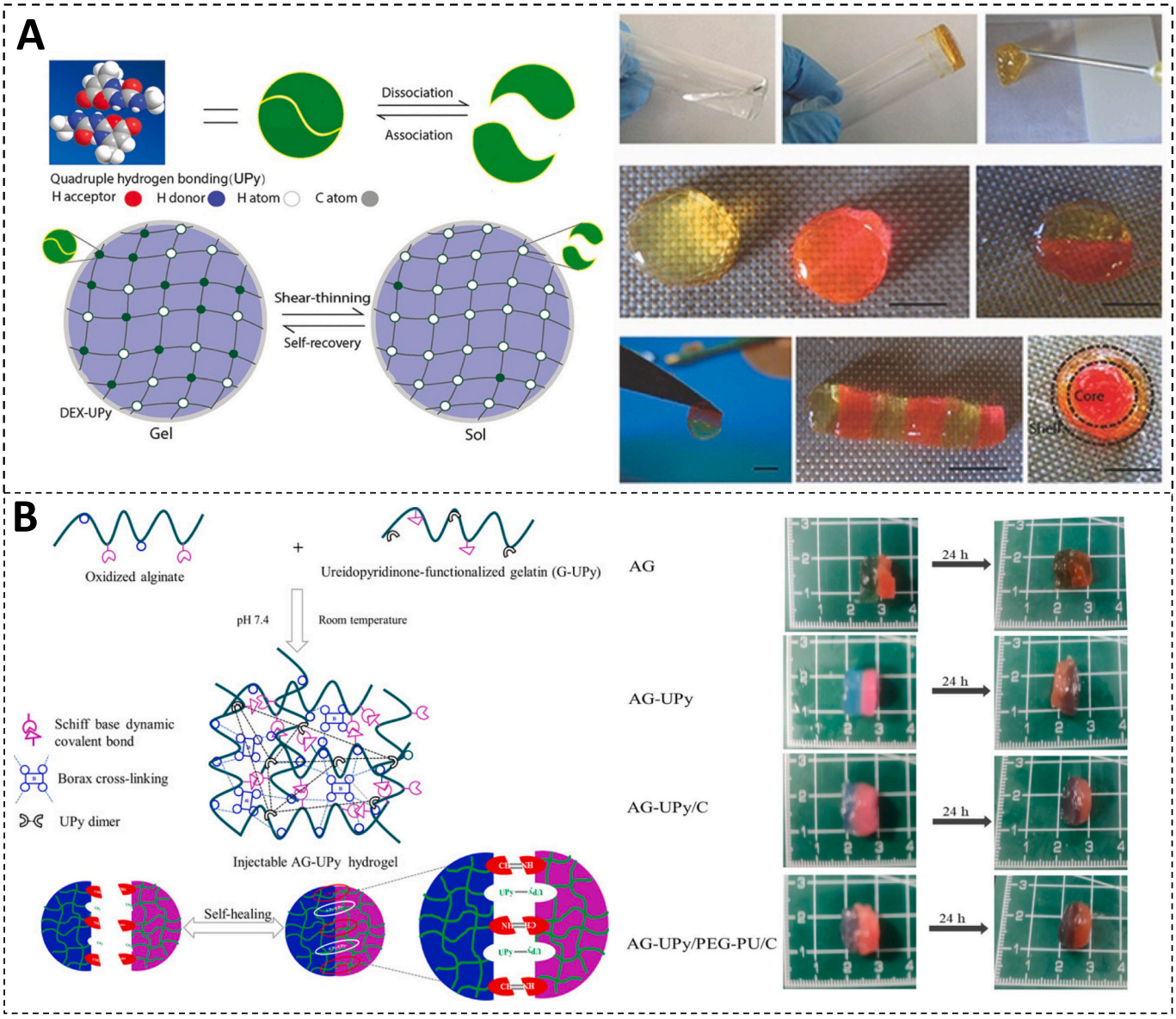

Fig. 6. Hydrogen bonding shear-thinning, self-healing mechanisms for cartilage tissue repair. A) Dextran-UPy system reproduced from [185] with permission from John Wiley and Sons. B) Oxidised Alginate-UPy, gelatin UPy system self-healed via dual mechanism Schiff base formation and UPy dimerization. Both hydrogels exhibit remarkable self-healing as shown by reintegration of separated hydrogels reproduced from [186] with permission from American Chemical Society.

plasma [192]. Recently, Li et al. have developed a shear-thinning, self-healing hydrogel based on (ADH)-modified poly(l-glutamic acid) (PLGA-ADH) and benzaldehyde-terminated poly(ethylene glycol) (PEG-CHO). PLGA-ADH and PEG-CHO precursor aqueous solutions could be injected via a dual-barrel syringe, resulting in the formation of PLGA/PEG hydrogels with excellent self-healing capability through dynamic reversible Schiff-base linkage between amino/hydrazide group on PLGA and aldehyde group on PEG. After rupturing, the groups quickly react with each other to form the new Schiff base bond again (Fig. 7B) [193].

4.3. Injectable systems with targeted defect adhesion

Typically, arthroscopies are carried out using air to distend the knee joint allowing for visualisation and surgical intervention. Unfortunately there have been some cases where the air has led to patients suffering from an embolism which have potential to be fatal [194,195]. As a result, some institutions have adopted water-filled arthroscopy which eliminates any risk of embolisms occurring. The importance of this is that many injectable hydrogel scaffolds are not able to be implemented under water filled arthroscopy as they will be washed away before gelation can even take place. Some recent efforts have focussed on developing hydrogels that can adhere to the cartilage surface to prevent being washed away as well as improving lateral integration with the native cartilage.

Utilising the Schiff-base bond in conjunction with photocrosslinking is a commonly used strategy for the design of hydrogels to help overcome the poor bonding strength of the Schiff-base bond by itself whilst also maintain the adhesion of the hydrogel to the defect for example Chen et al. functionalised HA with methacryloyl and aldehyde groups rendering it possible to crosslink via photopolymerisation and also form Schiff-base bands to the amine rich cartilage defect (Fig. 8A) [196]. An extremely promising system was recently designed by Hua et al. HAMA was used in combination with o-nitrobenzyl functionalised hyaluronic acid combined with gelatin (HANB/GL). After exposure to UV light, HAMA undergoes rapid photopolymerisation whilst simultaneously, HANB photogenerates aldehyde groups which form Schiff-base bonds with amino groups situated on gelatin and to the native cartilage interface. The hydrogel exhibited increased mechanical properties

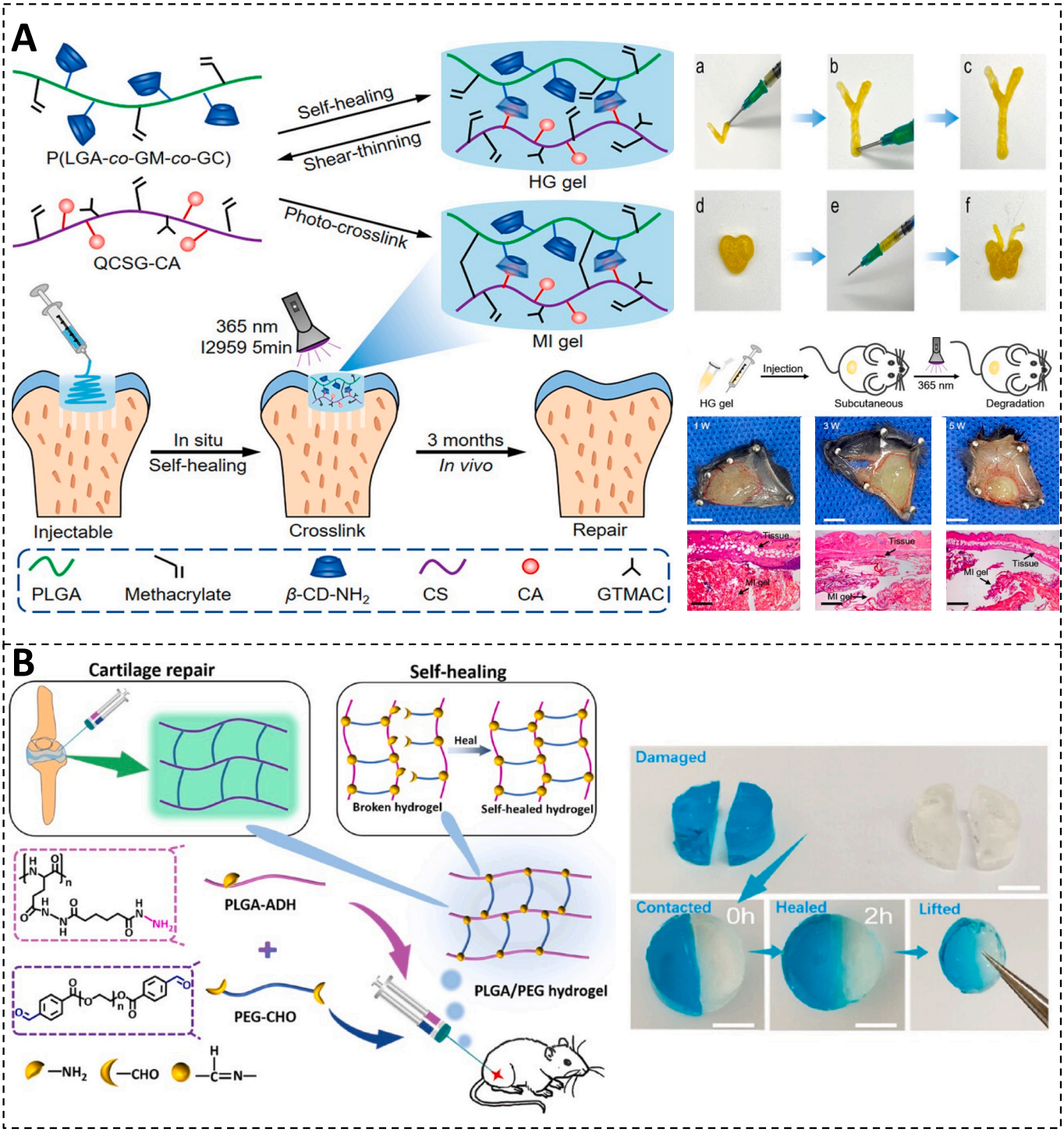

Fig. 7. Injectable shear thinning, self-healing hydrogel systems for cartilage repair displaying recovery after damage and removal of shear stress. A) A shear-thinning, self-healing injectable hydrogel system formed via host-guest interaction between cyclodextrin and cholic acid. The host material was composed of *β*-cyclodextrin and 2-hydroxyethyl methacrylate-modified poly(l-glutamic acid) (P(LGA-co-GM-co-GC)), while the guest material was chitosan modified by cholic acid, glycidyl methacrylate, and (2,3-epoxypropyl)trimethylammonium chloride. Reproduced from [189] with permission from American Chemical Society. B) A system composed of adipic dihydrazide (ADH)-modified poly(l-glutamic acid) (PLGA-ADH) and Dialdehyde functionalised poly(ethylene glycol) (PEG-CHO) via a Schiff base cross-linking reaction between amine and aldehyde groups on each respective polymer. Reproduced from [193] with permission from American Chemical Society.

relative to single networks composed of HAMA and HANB/GL alone whilst also possessing superior adhesive strength to fibrin glue: a clinically used tissue sealant (Fig. 8B) [197]. Whilst the use of Schiff-base bonds to provide adhesion have proved popular in cartilage tissue engineering, the bonds lack stability in aqueous environments and are prone to hemiacetal hydrolysis and glycoside-bond cleavage in acidic medium which is worrisome due to the presence of synovial fluid and its acidic pH in osteoarthritic joints [198,199].

Therefore, researchers have drawn inspiration from sea creatures like mussels, known for their remarkable ability to adhere to rough and wet surfaces. Their mechanism is based on the presence dihydroxyphenylalanine (DOPA) which contains a catechol group capable of

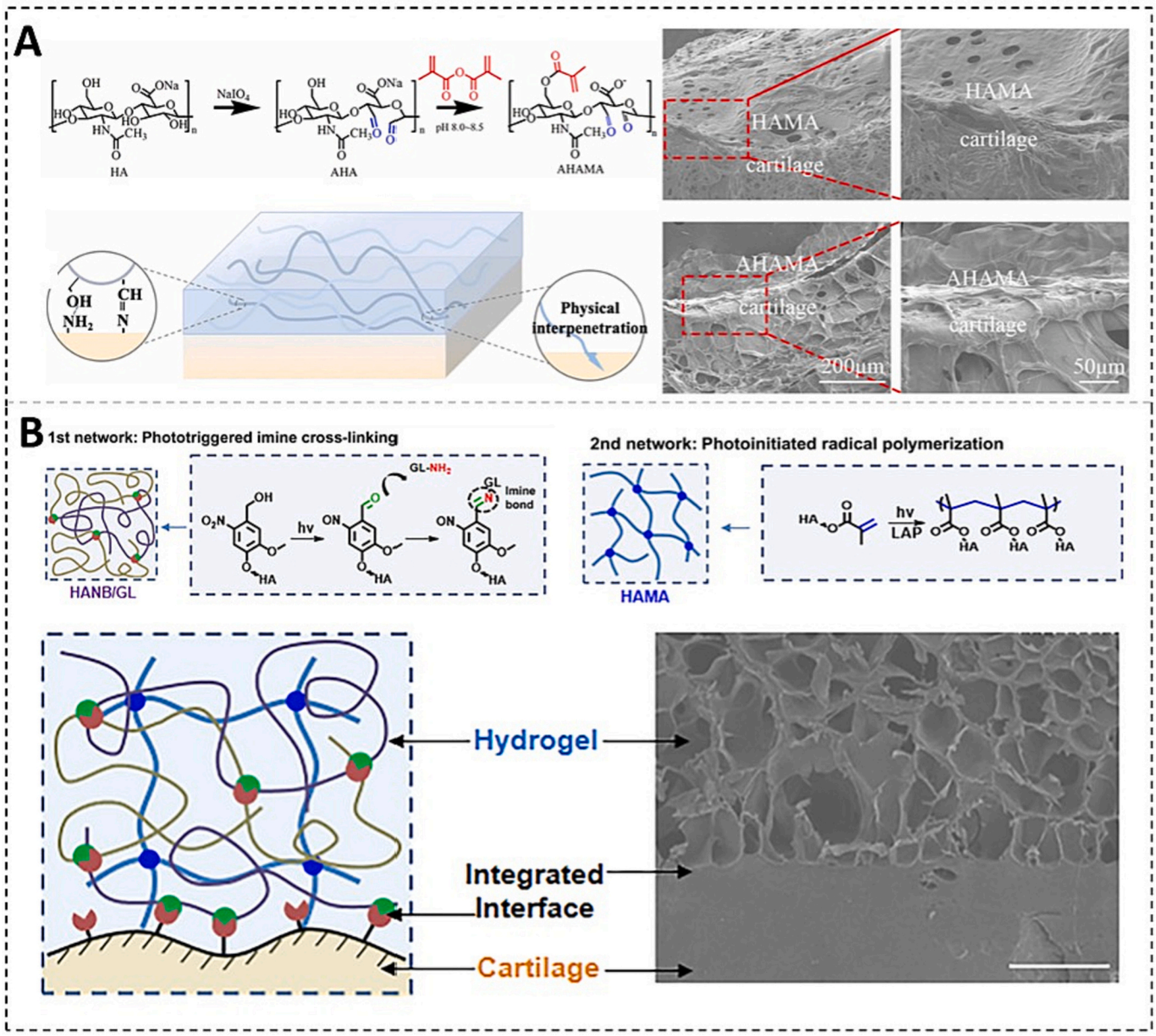

Fig. 8. Injectable hydrogels with targeted adhesion to the defect site by incorporation of chemical moieties. A) Aldehyde functionalised HAMA is photocrosslinked followed by Schiff-base bond *in situ*. Reproduced from [196] under Creative Commons license. B) A double network system composed of Norbornene functionalised HA (HANB), gelatin and HAMA. The system undergoes photocrosslinking whilst simultaneously aldehyde groups are photogenerated via NB therefore forming a Schiff-base bond to the amine rich defect. Reproduced from [197] under Creative Commons license.

binding to a variety of other molecules through physical and chemical interactions. Dopamine is an analogue of DOPA and capable of replicating the adhesive properties found on mussels and the presence of amine group on dopamine allows it to be easily functionalised onto polymers such as alginate, gelatin, HA, and CS [200–203]. Thereafter, these functionalised polymers are able to adhere to the wet cartilage surface taking advantage of the amine and sulphated nature of cartilage resulting from the high density of collagens and GAGs present (Fig. 9A). For example, Zhang et al. designed an injectable hydrogel composed of dopamine functionalised alginate, chitosan and silk fibroin whilst simultaneously delivering exosomes for endogenous BMSC recruitment. The hydrogel not only possessed a high adhesive strength of 120 kPa, but hydrogel promoted BMSC migration, proliferation, and differentiation. Notably, the adhesive hydrogel helped repair cartilage defects in rat patellar grooves by recruiting endogenous BMSCs into the defect via chemokine signalling pathways and inducing differentiation of BMSCs into chondrocytes (Fig. 9B) [204].

4.4. Hydrogel microparticles

An alternative solution to fabricate injectable hydrogels is to utilise pre-crosslinked micro and nano sized hydrogels as their size permits administration through a needle whilst also possessing shear-thinning behaviour. Typically, the size range of these particles is between 1-100 μm and are synthesised from a wide range of methods such as microfluidic emulsion, electrospraying, and mechanical fragmentation methods (Fig. 10) [13,205].

Once microparticles are injected into the defect, a secondary cross-linking mechanism takes place which essentially forms an assembled hydrogel system. These jammed hydrogel MPs can encapsulate cells and be administered via intra-articular injection with these systems also exhibiting shear-thinning and self-healing properties to facilitate

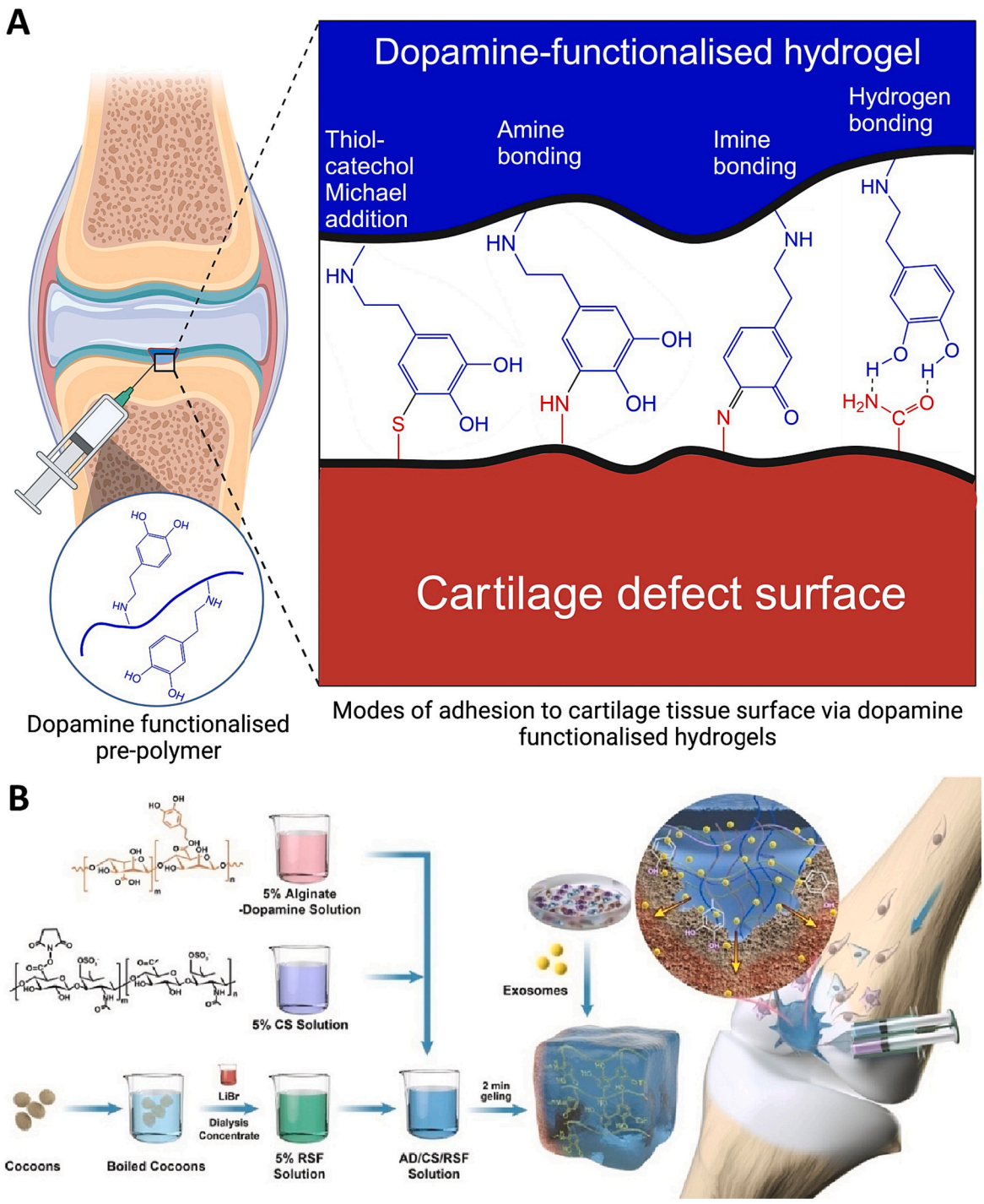

Fig. 9. A) Overview of mechanism of adhesiveness for dopamine functionalised hydrogel polymers (Created with Biorender.com). B) Alginate dopamine -Chitosan hydrogel provides mussel-inspired adhesion to the defect. Reproduced from [204] with permission from Elsevier.

injection [206]. Packing of the microgels into a defect forms a microporous network owing to interstitial voids between molecules which allows for more diffusion, transport of nutrients and waste and permeability (Fig. 11A) [13]. The porosity can be altered by tuning the size of the hydrogel MPs as well as the packing density which is independent of the matrix stiffness unlike bulk hydrogels, however if the size of the particle exceeds 10um, the hydrogels are considered colloidal rather than granular as their size permits influence from gravitational forces rather than thermal forces thus affecting their physiochemical properties [207]. Cells can move more freely through the porous structure, maximising cell interactions and enhancing synthesis of ECM [208,209].

Recent studies have shown granular hydrogel systems have been able to maintain chondrocyte phenotype and increase matrix synthesis in comparison to their bulk hydrogel system counterparts [210,211]. Zhu et al. designed a photo-annealed a granular hydrogel composed of hyaluronic acid, polyethylene glycol, and gelatin. Microparticles were formed and crosslinked by Diels-Alder reaction which could be mixed with chondrocytes and delivered to cartilage defects by injection, after which light was introduced to anneal the scaffold, leading to the formation of a stable and microporous chondrocyte deploying scaffold facilitating hyaline-like cartilage regeneration in a rat full-thickness cartilage defect model (Fig. 11B) [210].

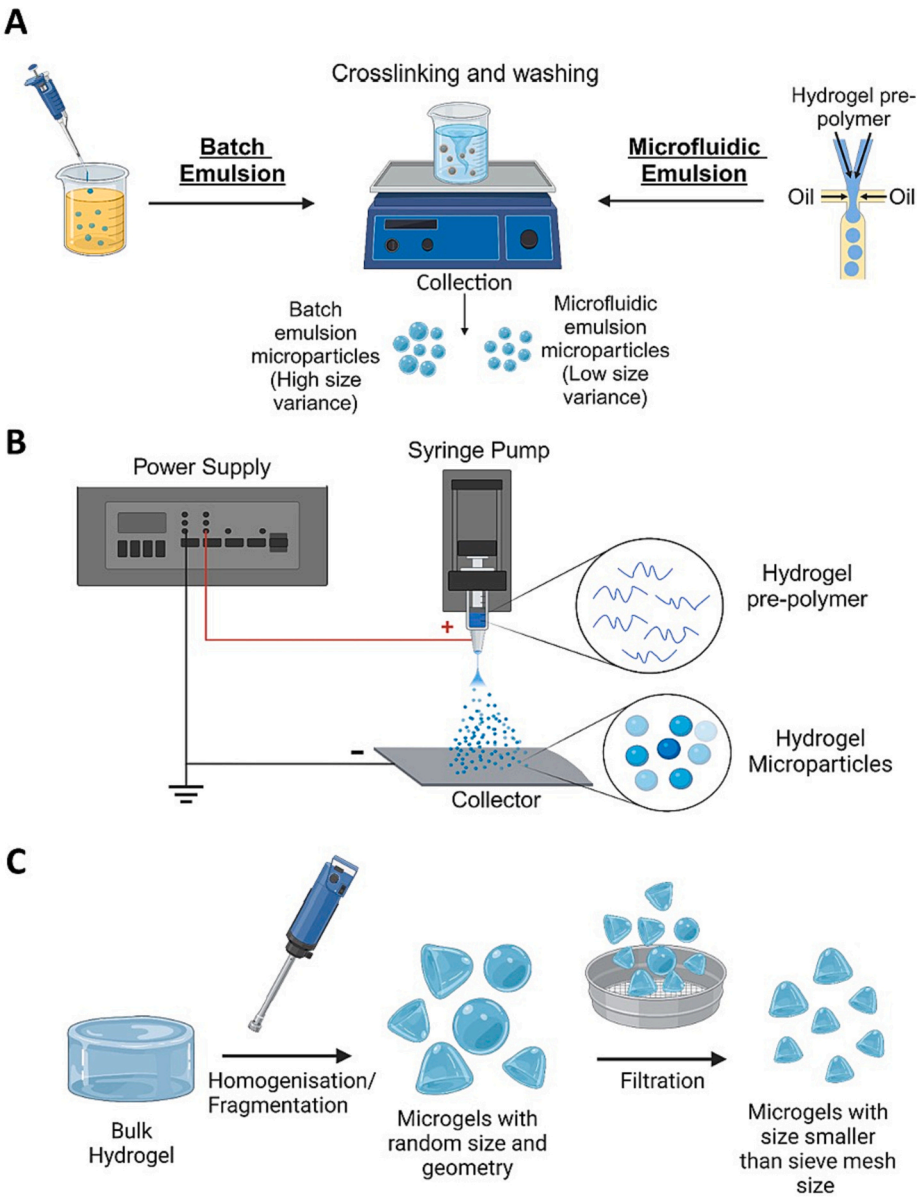

Fig. 10. Hydrogel microparticle fabrication methods. A) Schematic of emulsion techniques to generate microparticles. Batch emulsion technique forms particles with large size variance since droplet size is uncontrolled. Microfluidic emulsion limits the size of the particle to the microchannel size. B) Schematic of electrospraying to form microparticles. C) Mechanical fragmentation produces random microgels which can then be filtered using a sieve to provide a desired size. (Created with BioRender.com).

5. Hydrogels for therapeutic delivery

Whilst the scaffold material plays a significant role in the regeneration of cartilage through the process of cell-ECM interaction, there are various other signalling pathways mediated by numerous bioactive growth factors that play a role in regulating the process of chondrogenesis and hypertrophy. Therefore, encapsulation of these biologically active molecules inside hydrogels are very commonly used.

5.1. Growth factors

Growth factors are biologically active polypeptides produced natively and stimulate cellular division, growth, differentiation and regulation of articular cartilage homeostasis [212,213]. The transforming growth factor beta (TGFb) family, insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF) family and platelet-derived growth factor (PDGF) family all play an essential role in chondrogenesis, and hypertrophy thus have been exploited in cartilage tissue engineering to regenerate damaged articular cartilage. Bone morphogenetic proteins (BMPs) are members of the TGFb superfamily and have the ability induce formation of bone and cartilage, in particular BMP-2 and BMP-7 have shown their ability to innately stimulate chondrogenesis and ECM deposition [212]. However, growth factors typically have short half-lives in the range of minutes and are only activated upon proteolytic cleavage or binding to the ECM [214]. Utilising hydrogels as a means of delivering growth factors to provide spatial-temporal control is a promising strategy. Growth factors are typically incorporated into hydrogel scaffolds by direct loading, encapsulation into micro/nano carriers which are entrapped in the hydrogel or by covalent tethering [215,216].

PRP is rich in the aforementioned growth factors and is considered to initiate and regulate cartilage healing by stimulating cell proliferation and inducing chondrogenesis [217]. Conventional PRP therapy of intra-articular injection has been combined with injectable scaffolds to

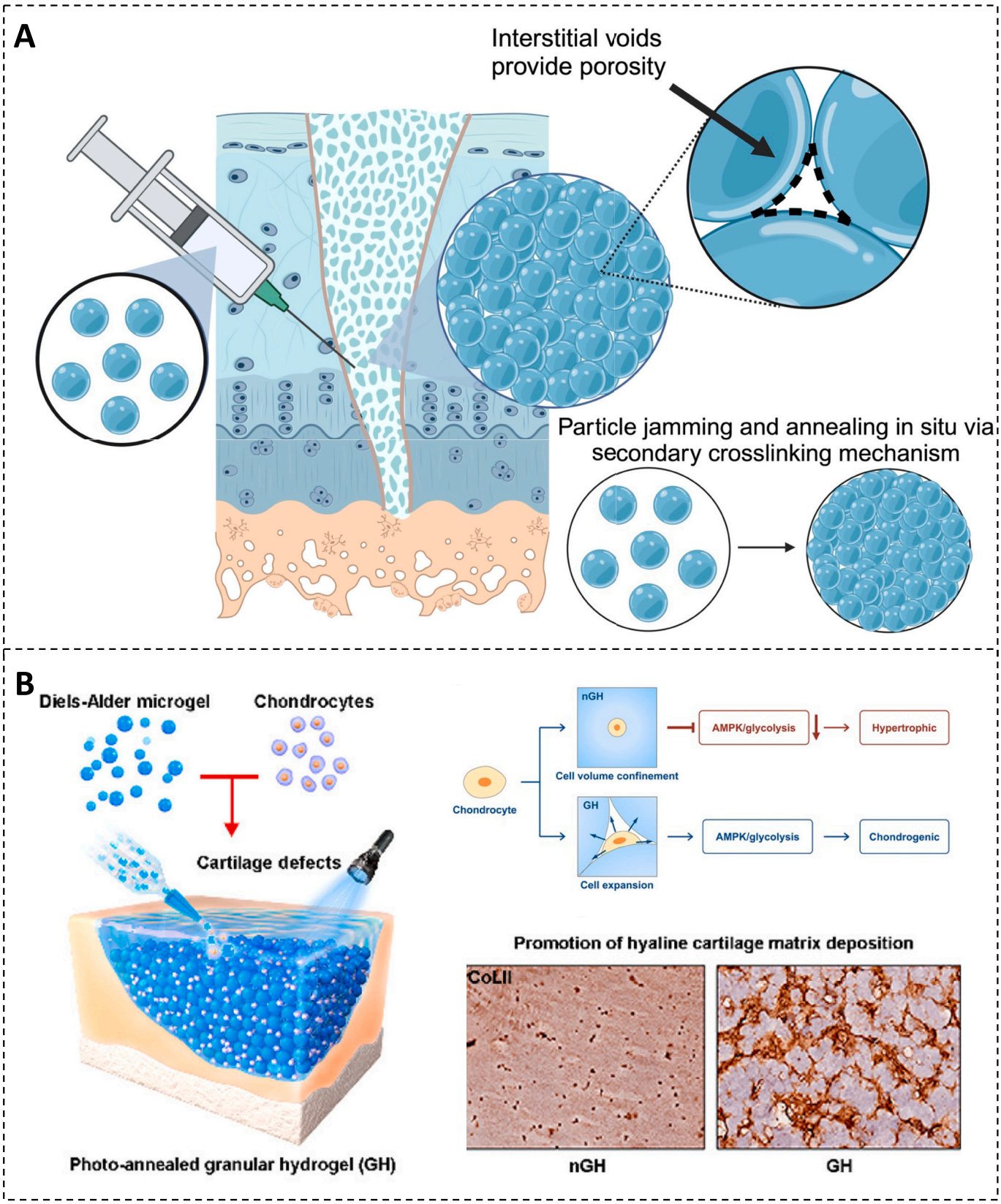

Fig. 11. A) Overview of granular hydrogels for cartilage repair. Pre-crosslinked microparticles are injected to defect followed by in situ annealing by secondary crosslinking. The resulting hydrogel possesses excellent mechanical properties while maintaining microporosity conducive for cells (Created with BioRender.com). B) Diels-alder formed microparticles annealed by photopolymerization possessing superior chondrogenesis compared to a conventional bulk hydrogel system composed of the same polymers, Reproduced from [210] with permission from American Chemical Society.

provide a more sustained release; however, these systems suffer from the need for surgical implantation. As a result, Liu et al. produced a photoinduced, imine crosslinked, hydrogel based on hyaluronic acid functionalised by o-nitrobenzyl alcohol. Under light irradiation, aldehyde groups are formed which can bond to amino groups on PRP as well as to the tissue surface; therefore, the in situ forming hydrogel had excellent adhesive properties and was shown to provide a controlled release of growth factors in vivo [218]. A similar product to PRP is platelet lysate. Compared to PRP which consists of blood platelets containing growth factors necessary for chondrogenesis, instead, platelet lysate contains the lysate formed via repetitive freeze-thaw cycles of PRP and further centrifugations providing a high concentration of growth factors making it another commonly used molecule for cartilage tissue engineering hydrogels. Injectable hydrogels loaded with growth factors or platelet products are detailed in Table 2.

5.2. Kartogenin

Kartogenin (KGN) is a small bioactive molecule reported to enhance the differentiation of MSCs into chondrocytes. First identified by Johnson et al. KGN not only maintained the chondrogenic phenotype but also possesses chondroprotective abilities by inhibiting matrix breakdown by MMPs and is therefore promising for slowing the progression of OA [235,236]. Kartogenin has seen promising results ex vivo and on in vivo animal models by promoting Type II collagen and aggrecan synthesis and regulating catabolic activity and inflammation [237,238]. Recently, Johnson et al. have developed an analogue of Kartogenin (KA34) which underwent a phase I clinical trial which reported KA34 as a safe OA drug candidate with disease modifying, cartilage regenerative and pain modulating activities [239]. Nonetheless, KGNs size and low water solubility limits the therapeutic application of KGN. As a result, utilising KGN with hydrogel carriers has alleviated concerns with fast clearance of the small molecule from the synovial joint [218–220].

5.3. NSAIDs and corticosteroids

Although rheumatoid arthritis (RA) and OA are inherently different diseases, inflammation is still a characteristic of early OA and an inherent source of pain, thus methods for anti-inflammatory drug delivery in RA can be translated to OA. Conventional intra-articular injection of NSAIDs and steroids are limited by fast clearance by synovial fluid whereby drugs only reside in the joint for a few hours post IA injection [240,241]. To overcome this short release time, combining these therapeutics with injectable hydrogels is hypothesised to sustain the release time. Table 3 summarizes hydrogel drug delivery systems that have been coupled with NSAIDs or steroids [224].

Table 2
Recent progress in delivery of growth factors with injectable hydrogels.

Growth Factors	Method of injectability / Crosslinking	Hydrogel material	Loading mechanism	Release rate	Ref.
TGF-b1	In situ gelling/ thermosensitive	Silk fibroin blended with polylysine modified chitosan/GP	Adsorption	~20-50% after 28 days	[219]
	In situ gelling/ thermosensitive	Thiolated chitosan and carboxymethyl cellulose hydrogel	Adsorption	19-81% after 21 days	[220]
	Enzymatic crosslinking	Collagen-TA, HA-TA	Adsorption	Not reported	[159]
	In situ gelling / thermosensitive	Collagen hydrogel – PLGA microparticle composite	TGF-b1 loaded PLGA microparticles encapsulated into bulk hydrogel	~100% release after 21 days	[221]
	Dual crosslinked in situ gelling: 1. Thermosensitive 2. Photocrosslinking	Glycidyl methacrylate-modified hydroxypropyl chitin	Adsorption	~70% release after 72 hours	[222]
TGF-b3	In situ gelling / thermosensitive	Growth factor loaded PLGA MPs encapsulated in methoxy poly(ethylene glycol)-poly(alanine)	Tgf-b3 loaded into PLGA MPs encapsulated into bulk hydrogel	~60% after 2 months	[223]
	Photocrosslinking	Encapsulation in photocrosslinkable aldehyde-methacrylate functionalised alginate and amino-gelatin.	adsorption	Not directly reported, ~100% release of BSA after 30 days	[224,225]
	Michael addition	POSS-PEEP-thiolated HA hydrogel	Adsorption	86.1% after 15 days	[226]
PDGF	Granular hydrogel (microislands), 1. Photocrosslinking 2. Annealed by enzymatic crosslinking	HAMA and sulphated HAMA	Electrostatic interaction between -ve SHAMA and +ve TGFb3 and PDGF	20% TGF-b3 released after 2 weeks 2% PDGF release after 2 weeks	[205]
	Microparticle-Hydrogel composite	Alginate micropsheres then loaded into a silk-chitosan hydrogel	Core-shell microparticle: KGN loaded into core. PDGF loaded into shell	70-90% after 7 days	[227]
BMP-2	Thermosensitive	BMP-loaded PLGA microspheres encapsulated withing P127 hydrogel	BMP loaded into MPs encapsulated within bulk hydrogel	~90-95% released after 10 days	[228]
IGF	Photocrosslinking	GelMA loaded with IGF-mimicking nanofibers	IGF-mimicking nanofibers loaded into bulk hydrogel	Not reported (in vivo study)	[229,230]
PRP	Ionic crosslinking	Alginate crosslinked by calcium ions released from $CaCO_3$ -GDL	Adsorption	Not reported	[231]
	Schiff-base	PRP loaded HA-ALH, HA-ADH hydrogel and MnO2 nanoparticles loaded with BSA	Adsorption	~70% proteins release after 3 days and ~125pg/ml of TGF-b. pH dependent	[192]
Platelet lysate	Enzymatic crosslinking	HA-TA	Adsorption	Not reported	[232]
	In situ gelling, thermosensitive	PDLLA-PEG-PDLLA	Adsorption	Total protein release varied from ~50-90% after 40 days based on degrading enzyme	[233]
	Electrostatic interactions	Chitosan – chondroitin sulphate hydrogel NPs	PL loaded NPs	61% and 71% released after 7 days for Tgf-b1 and PDGF respectively	[234]

Table 3
Injectable hydrogel systems used to deliver NSAIDs or steroids for symptomatic joint pain relief.

Classification of Drug	Drug	Hydrogel	Mechanism of delivery	Release time	Ref.
NSAIDs	Celecoxib	Thermo-responsive acetylated PCLA-PEG-PCLA	Erosion of hydrogel	~4 weeks	[242,243]
	Naproxen	Schiff base (dextran (Dextran-ox), gelatin and hyaluronic acid)	Diffusion and degradation of hydrogel	240 mins	[244]
	Meloxicam	Drug loaded NPs encapsulated inside carboxymethyl chitosan, methylcellulose, pluronic and zinc chloride hydrogel	Bulk and surface erosion of NPs followed by diffusion of drug from hydrogel matrix	37 Days	[245]
	Diclofenac	Thermoresponsive HA - Poloxamer 407	Diffusion	40% release after 4 days	[246]
		GelMA microspheres coated with (DMA-MPC)	Degradation of hydrogel network	~12-20% in 2 days	[247]
	Piroxicam	Thermoresponsive HA – Poloxamer 407	Diffusion mediated by highly packed super-molecular structure of poloxamer 407	10 days	[248]
	Ibuprofen	PEG microspheres	Hydrolysis of the ester link between the drug and microsphere	~2% after 3 months	[249]
Steroids	Triamcinolone acetonide	Thermo-sensitive poly(organophosphazene) microspheres	Degradation of the hydrogel network to overcome hydrophobic interaction between hydrogel and drug	6 weeks	[250]
		Drug loaded PLA/PEG-PDL MPs loaded into thermoresponsive poly(PEGMA) hydrogel	Erosion/relaxation of hydrogel network and diffusion	~60% release in 24 hours	[251]
	Dexamethasone	Schiff base (dextran (Dex-ox), gelatin and hyaluronic acid)	Diffusion and degradation of the hydrogel	~22% after 5 days	[244]
		Drug-loaded PLGA MPs encapsulated in chondrocyte laden agarose hydrogel	Diffusion and degradation of hydrogel	~33% after 28 days	[252]
		Drug loaded PLGA NPs encapsulated in four-arm maleimide-functionalised PEG	Cleavage of MMP degradable crosslinks	Not reported (release varied depending on concentration of collagenase used)	[253]

As discussed, hydrogels possess excellent capabilities to perform as scaffolds, facilitating essential ECM-cell interactions. At the same time, they can concurrently serve as delivery vehicles for cells, drugs, and therapeutics. Conventional intra-articular (IA) injections offer temporary relief and symptomatic treatment but are limited by the short half-life of therapeutic agents and lack of targeted delivery, which hinders their overall efficacy [254]. In contrast, injectable hydrogels can function as drug-delivering scaffolds, enabling sustained and localized drug release through various encapsulation methods. Therapeutics can be easily encapsulated within the crosslinked network formed by hydrophilic polymer chains commonly known as the mesh. The release of physically encapsulated cargo is mediated by rate of diffusion which is dependent on the mesh size, degradation rate, swelling ratio, and drug size (Fig. 12A) [255–257]. As the mesh size and drug size converge, the biological molecule becomes further entrapped until the point of immobilisation. Immobilisation may also be achieved by having hydrogel-cargo interaction i.e. physical or chemical crosslinking of the therapeutic to hydrogel polymeric chains (Fig. 12B). To release immobilised cargo, the hydrogel requires disruption to the matrix via swelling or release mediated by external stimuli, in addition to enzymatic/hydrolytic degradation of the matrix to release the therapeutic from the hydrogel [258–260]. Micro/nanoparticles may also be employed for cargo delivery but their small surface area to volume ratio means they are also limited by fast diffusivity of their cargo [261]. To overcome this, micro/nanoparticles can be combined with bulk hydrogels in a composite system, thereby requiring the degradation of two hydrogel matrixes for release to occur, thereby providing further sustainment. By capitalizing on these mechanisms, injectable hydrogels hold tremendous potential for enhancing cartilage repair compared to conventional IA therapies [262].

The mechanism of loading drugs into the hydrogel plays a major role in the release mechanism of the therapeutic agent. To encapsulate drugs within the hydrogel network, the drug solution can be mixed with the precursor hydrogel solution and upon polymerisation, the drug molecules become physically trapped within the matrix. The diffusivity is altered by polymeric chains in the hydrogel network with open spaces between the chains known as mesh [264]. The mesh size as well as the molecular size of the drug determines diffusivity (D) through a hydrogel owing to steric interactions between the drug and polymeric network. If the mesh size, η , is relatively larger than the size of a drug molecule, r_{drug}, then the dominant mechanism of release is diffusion and can be quantified by Stokes-Einstein equation [257].

$$D = \frac{RT}{6\,\pi\,\eta\,r_{drug}} \tag{1}$$

Where R is the gas constant and T is the absolute temperature. As the size of the drug increases relative to the size of the mesh, the rate of diffusion decreases until the drug is fully entrapped and immobilized within the matrix. Subsequently, swelling, mechanical deformation, or mechanical degradation of the network is required to facilitate the release of entrapped drugs.

As an alternative to direct encapsulation, the hydrogel may be specifically designed such that it has an affinity with the therapeutic molecule, either by covalent linkage or physical interactions (electrostatic, hydrophobic). Compared to direct encapsulation, the release of drugs when these drug-hydrogel interactions are involved are independent of the mesh size and drug molecule size and are only released after covalent linkages have been cleaved or degradation of the hydrogel network. For example, TGFb was thiolated and covalently incorporated into PEG diacrylate (PEGDA) hydrogels which was shown to promote chondrogenesis and differentiation of MSCs [265,266]. Covalent conjugation of TGF-b1 to the hydrogels provide a more favourable microenvironment to induce chondrogenesis when compared to hydrogels with TGF-b1 incorporated via adsorption [267]. Furthermore, KGN possesses carboxyl moieties allowing for covalent conjugation to polymeric networks which can further sustain the release profile of this small molecule. For example, the amino group in chitosan is able to covalently bind with the carboxyl group of KGN showing an exceptional increase in release rate of up to seven weeks compared to just three weeks without covalent binding [225,268,269]. Bedouet et al. designed PEG microspheres which exhibited extremely slow release of ibuprofen as a result of hydrolysis of a drug-hydrogel ester linkage [249]. Puiggali-Jou et al. took advantage of positively charged growth factors TGF-3 and PDGF and loaded them into HAMA which was sulphated thereby giving the hydrogel a negative charge to generate electrostatic interaction between the growth factors and the hydrogel. The hydrogel was then

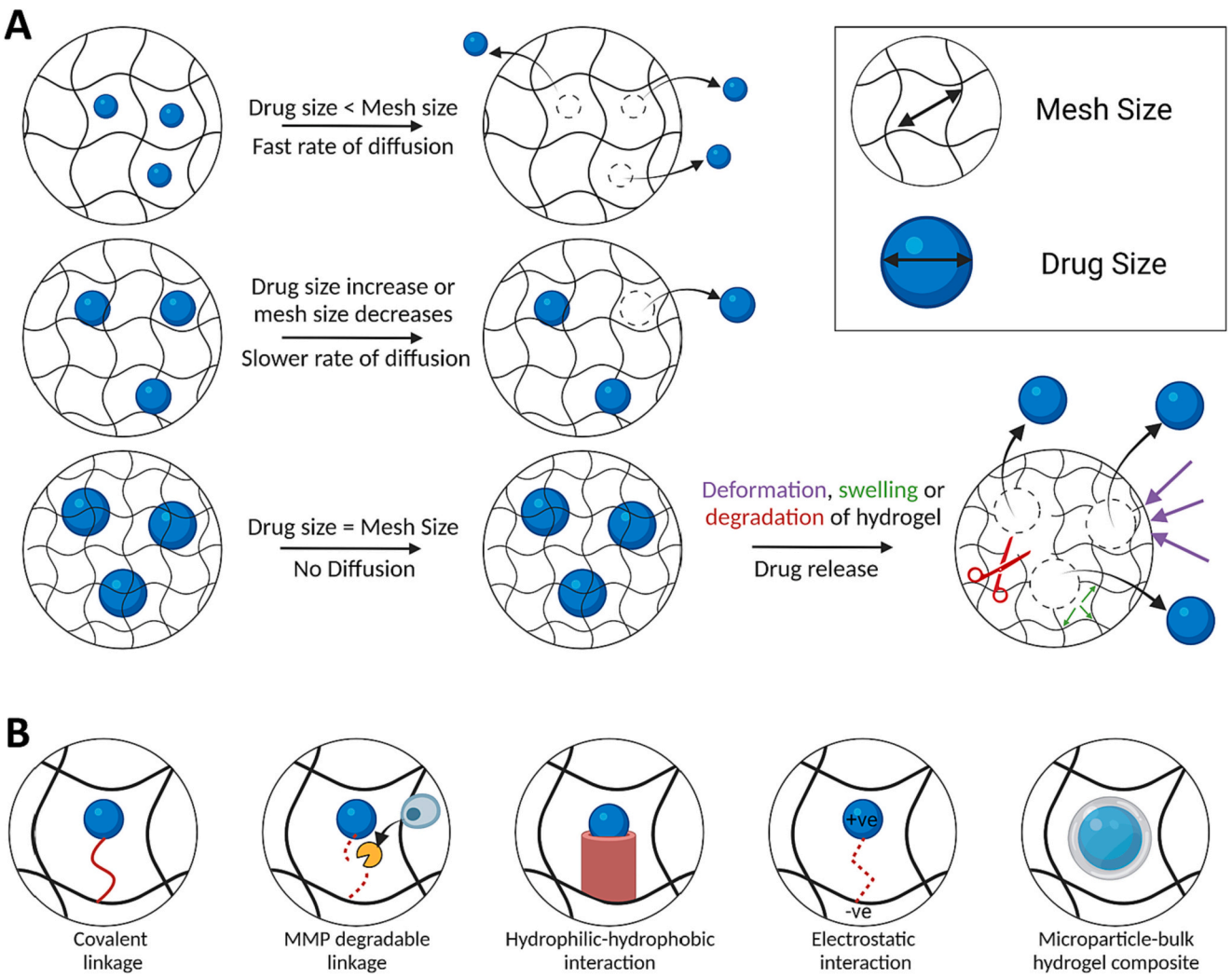

Fig. 12. A) Mechanism of release for altered size of drug and/or mesh size. As drug size increases or mesh size decreases leading to increased degree of drug entrapment rate of diffusion slows down. In the case of immobilisation (drug size > mesh size) there is a requirement for degradation, deformation or swelling of the hydrogel mesh is required to release entrapped drugs. B) Depending on the hydrogel and therapeutic being utilised, specific interactions between the drug and hydrogel can be engineered which has the benefit of further sustaining the release of the therapeutic. Redrawn from [263] (Created with Biorender.com).

fragmented into "microislands" to form a granular hydrogel annealed via enzymatic crosslinking in situ with the system showing increased matrix deposition and cartilage tissue maturation when compared to bulk or homogeneous granular hydrogels whilst the sustained release of growth factors provided biochemical cues for guiding cell migration and differentiation into cartilage [211].

In the native cartilage ECM, cells are responsible for mediating tissue development, enabling tissue composition and structure to evolve during growth and development [270]. The native ECM is degraded by MMPs and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) which are secreted by chondrocytes. Hydrogels with enzyme-sensitive peptide crosslinks can undergo cell-mediated degradation and therefore degradation rate of the scaffold can be tuned finely with ECM synthesis, but this network degradation may also permit the release of therapeutics that are tethered to the hydrogel by enzyme-degradable crosslinks [266,271,272]. MMP-13 is particularly related to the degradation of articular cartilage in OA by aggressive breakdown of Type II collagen and it has been shown that patients who have suffered AC defects have upregulation of MMP-13 [273]. Recently, Tianyuan et al. took advantage of this biochemical cue by designing a PEG hydrogel with MMP-13 degradable peptides via Michael addition, when loaded with KGN and exosomes in vitro and in vivo studies show that the hydrogel possessed good injectability, on-demand anti-inflammation, and immunomodulation capabilities [274]. A four-arm maleimide-functionalized polyethylene glycol (PEG-4MAL) encapsulated PLGA NPs loaded with dexamethasone. Not only did the system possess excellent mechanical properties but the hydrogel was synthesised with crosslinks formed commonly expressed during OA. As a result, the hydrogel system degraded in coherence with the progression of OA and subsequently released dexamethasone on-demand [253].

Alternatively, the release of therapeutics through network degradation can be facilitated through external stimuli to provide on-demand release [275]. Magnetic fields can cause encapsulated magnetic nanoparticles to deform the hydrogel matrix, sonication with an ultrasound transducer to cause network degradation as well as UV light triggered degradation via photolysis [276,277]. Cogan et al. utilised an alginate hydrogel loaded with phospholipid vesicles which had thermos-responsive crosslinks to magnetic nanoparticles. Upon exposure to an electromagnetic field, vesicle content was released inducing a cellular response leading to the increased expression of collagen secreted by chondrocytes after 14 days relative to hydrogels without electromagnetic stimulation. (Fig. 13A) [278]. Yuan et al. prepared an injectable chitosan-chondroitin sulphate hydrogel via Schiff base reaction and PLGA MPs which were loaded with KGN and embedded within the hydrogel. Results showed that the MPs increased the compressive modulus of the hydrogel but also, following sonication with an

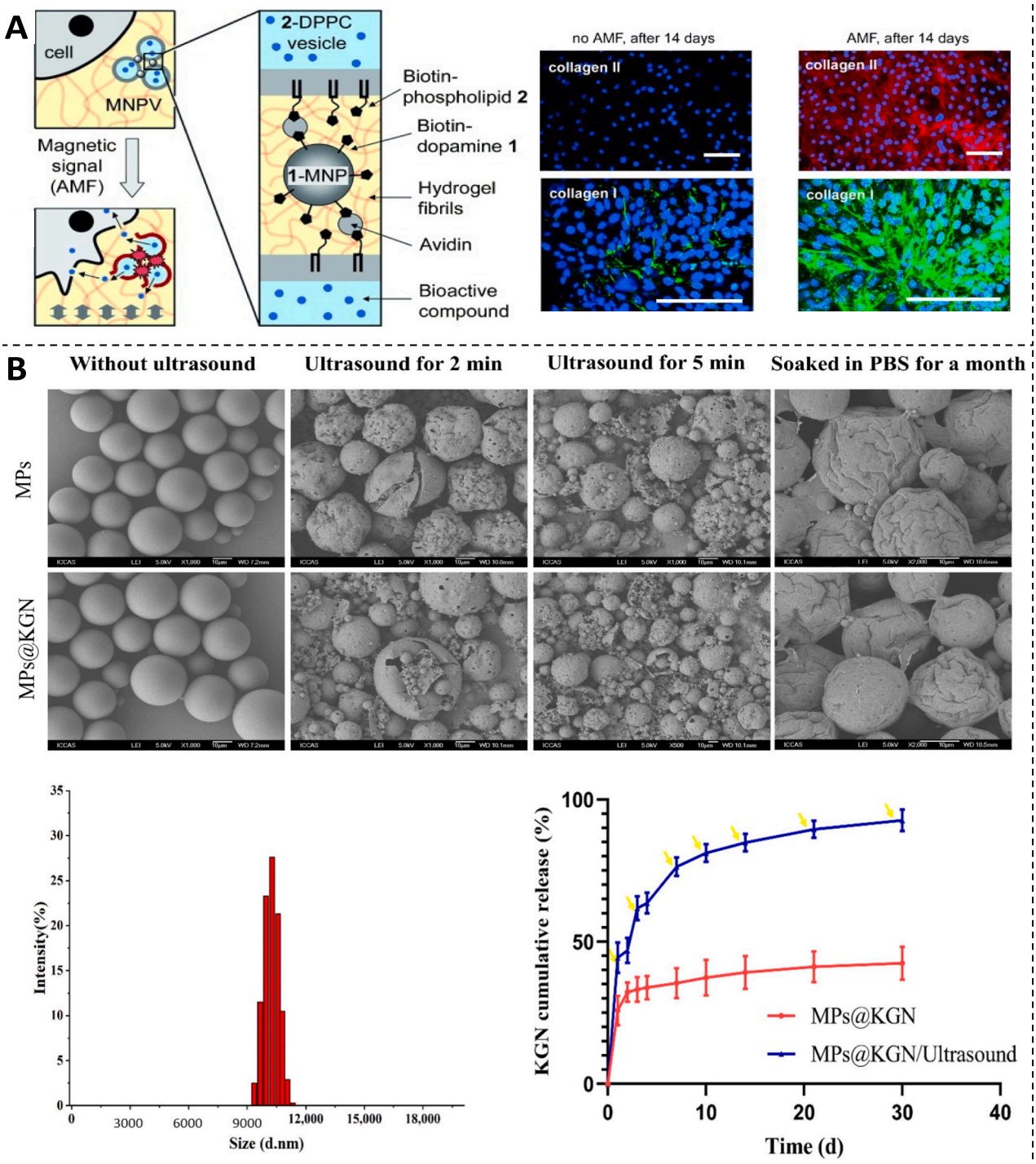

Fig. 13. External stimulation of hydrogels to release encapsulated therapeutics. A) Cells and magnetic nanoparticle-vesicles (MNPVs) are co-immobilized within an alginate hydrogel (yellow). MNPVs are self-assembled nanocarriers composed of magnetic nanoparticles coated with N-biotinoyl dopamine 1 (1-MNP) and DPPC vesicles containing biotin-DHPE 2 (2-DPPC), which are linked together by avidin. Biologically active molecules, such as drugs (blue), can be non-invasively released by an alternating magnetic field (AMF), and these released therapeutics in turn induce responses from cultured cells upregulation collagen expression in chondrocytes. Reproduced from [278] with permission from John Wiley and Sons. B) Chitosan-chondroitin sulphate microspheres encapsulating KGN exhibited release which was mediated by ultrasound transduction. Reproduced under Creative Commons license. [277].

ultrasound transducer, the burst release of KGN could be controlled (Fig. 13B) [277]. One significant drawback of external mechanical stimulation for drug release is that the hydrogel network may undesirably degrade during transduction. To overcome this, the use of self-healing hydrogels for these drug delivery systems is preferential.

A major challenge for therapeutic delivery to cartilage is the miniscule pore size (~6-14 nm) of the ECM [16,279]. As a result, only very small drugs are even able to penetrate the cartilage matrix but again this is limited by the fast clearance of these drugs from the synovial joint. As a result, smart hydrogel micro-/nanocarriers have the potential to overcome the obstacles posed by the dense structure of the cartilage matrix. Micro-/nano-carriers possess the innate ability to have their surface modified by physical and chemical interactions to not only increase their adhesion to the defect site but to also increase their

lubrication, a property that is so often neglected during the design of injectable hydrogels [280]. Lei et al. designed HAMA microcarriers functionalised with cationic liposomes loaded with rapamycin with the for specific cartilage targeting via electrostatic interaction whilst synergistically providing enhanced lubrication properties due to self-renewable hydration layers (Fig. 14A) [281]. Han et al. utilised a dopamine and 2-methacryloyloxyethyl phosphorylcholine coating which gave the microspheres cartilage adhesive and lubricating properties respectively [247]. In a similar manner, Lin et al. utilised dopamine modified HAMA microspheres to adhere to the cartilage surface but were also encapsulated with gallic acid-loaded liposomes as a secondary structure which was positively charged allowing for enhanced diffusion through the dense cartilage matrix (Fig. 14B) [282]. Yu et al. took advantage of the cartilage ECM by utilising a nanoparticle encapsulating KGN and Dex whilst also incorporating a Type II collagen targeting peptide for specific targeting of cartilage [283]. All of which are promising system to provide solutions for overcoming the poor penetration of therapeutics through the cartilage matrix.

6. Clinical translation of hydrogels for cartilage repair

Advances in the field of cartilage tissue engineering have led to the development of many hydrogel systems, many of which are described in this paper. However, only a few injectable hydrogels are commercially available due to the lengthy and costly regulatory approval requirements. Since there are only a few commercially available injectable hydrogels scaffolds are available for the purpose of cartilage tissue engineering, hydrogels that are currently in clinical trials are also included (Table 4). These injectable hydrogels can be categorised as hydrogels used as augmentation for microfracture procedures (e.g. JointRep®, BST-CarGel®, GelrinC™, ChonDux and CARTISTEM®) augmentation for ACI procedure (e.g. NOVOCART® Inject plus) and hydrogels for the repair and symptomatic treatment of cartilage lesions (Hy2Care®, Arthrosamid®).

The injectable hydrogels used as augmentation strategies for microfracture are effectively iterations of the AMIC procedure first introduced by Behrens whereby a collagen I/III matrix protects the blood clot and enhances chondrogenesis [304]. The use of injectable hydrogels such as JointRep®, BST-CarGel®, Gelrin C™, ChonDux and CARTISTEM® provides increased ease of implementation as compared to the traditional collagen matrices. JointRep® is a thermosensitive hydrogel composed of deacylated chitosan which is cationic providing it with excellent adhesive ability. A clinical study showed admirable results when used in conjunction with microfracture whereby the WOMAC score decreased by 88% at 6 months and 93% at 12 month follow up stages and post-op study group showed Type II collagen and hyaline-like cartilage in the regenerated tissue [286]. Similarly, BST-CarGel® is also a themosensitive chitosan-based hydrogel but requires mixing with the patient's whole blood before surgery which has the benefit of reinforcing the clot during coagulation and impeding its retraction [305]. Gelrin C™ is based on PEGDA and denatured fibrinogen and is injected in liquid form to the defect directly after the microfracture procedure. Following 90 seconds of exposure to UVA light, the defect is filled with the formed hydrogel. A 24-month follow up of GelrinC™ applied to 56 patients showed the significant increase of MOCART score, likely transformation of repair tissue to hyaline cartilage and effectively supports the potential of GelrinC™ as a treatment type for chondral and osteochondral lesions [306]. Similar to GelrinC™, ChonDux is also based on PEGDA but incorporates HA and CS to develop a fast-gelling bioadhesive. Data from a clinical trial is supporting the CE approval of the product although a 2-year follow up showed ChonDux promotes stable restoration of full thickness articular cartilage defects [301]. CARTISTEM® utilises a HA hydrogel at 4% but is the only hydrogel system utilising an allogenous cell source (human umbilical cord derived MSCs at 5 million cells/ml of hydrogel). A 7-year follow up of the phase I/II trial showed CARTISTEM® to be a safe and efficacious treatment modality for patients with OA related chondral defects. The phase III trial compared the system with conventional microfracture, with the follow up across 5-years showing CARTISTEM® to have significantly improved cartilage repair compared to conventional microfracture [302,303].

NOVOCART® Inject plus is an augmentation strategy for ACI. Like traditional ACI, chondrocytes are harvested arthroscopically, expanded, and combined with NOVOCART® Inject plus which is composed of several components namely expanded chondrocytes, albumin and hyaluronic acid. A dual-chamber syringe with the cell suspension and hydrogel components in one chamber and the hydrogel crosslinker in the other chamber is used to completely cover the defect. The hydrogel is currently in phase III study with a 5-year follow-up time to treat 100 patients with chondral or osteochondral defects, a recent analysis at a 2-year time point has shown 93% of patients had at-least a 10% increase on their KOOS score compared to pre-operative level and MRI analysis showed increased maturation, reorganisation, and integration of repair tissue [307].

Hy2Care® and Arthrosamid® are both standalone hydrogels that are not part of any augmentation strategy for microfracture or ACI. Hy2Care® is an enzymatically crosslinked hydrogel based on hyaluronic acid and dextran. A dual chamber syringe system is utilised where dextran and hyaluronic acid which are situated in their own chamber mix as they exit the syringe and form a hydrogel in situ within 30-40 seconds. Whilst the hydrogel can be administered via IA injection, the first clinical study will be performed in a mini-open procedure, and it is hoped that the data obtained from the clinical trial will aid the CE application of this product [292,293]. Arthrosamid® is described as a non-biodegradable hydrogel composed of polyacrylamide (2.5%) and non-pyrogenic water (97.5%) providing symptomatic treatment for knee osteoarthritis. The hydrogel is injected into the joint cavity which cushions the joint to reduce pain, decrease stiffness and aid movement. Whilst the hydrogel is expected to increase viscosity of synovial fluid and improve lubrication, the product separates itself from other viscosupplementation injections since it becomes part of the soft synovial tissue in the joint capsule. A limitation of Arthrosamid® is that the polyacrylamide material reportedly has some cause for controversy. A polyacrylamide viscosupplement from Noltrex®, Russia caused host tissue reaction in the form of foreign body granuloma, edema, inflammation, and redness induration [308]. Although the case was reported as a unique adverse event and the cause unclear, the use of polyacrylamide warrants careful consideration.

7. Conclusions and future perspectives

In this comprehensive review, we explored the recent strides made in the design and development of injectable hydrogels for minimally invasive cartilage repair. Compared to conventional procedures, the use of injectable hydrogels offers numerous advantages, including reduced surgical invasiveness, the ability to fill large and irregular defects, and simplified implementation compared to the complex suturing and gluing required for pre-formed matrices. Moreover, these hydrogels hold immense potential as delivery vehicles for biologically active molecules, such as cells, growth factors, steroids, and NSAIDs, addressing the limitations of standalone therapeutic injections with fast release, repetitive administration, and clearance.

Despite the promising potential of injectable hydrogel-based cartilage repair, several challenges persist, hindering their widespread clinical application. Among these challenges is the need to achieve mechanical properties in hydrogels that closely match those of native cartilage. Given the complex nature of cartilage as a load-bearing tissue with unique viscoelastic properties, hydrogels often fall short in terms of stiffness and resilience, especially in defects that are likely to be situated on weight-bearing portions of the cartilage, leading to sub-optimal regeneration and potential compromise of overall joint function.

Long-term stability is another crucial concern, as certain hydrogels

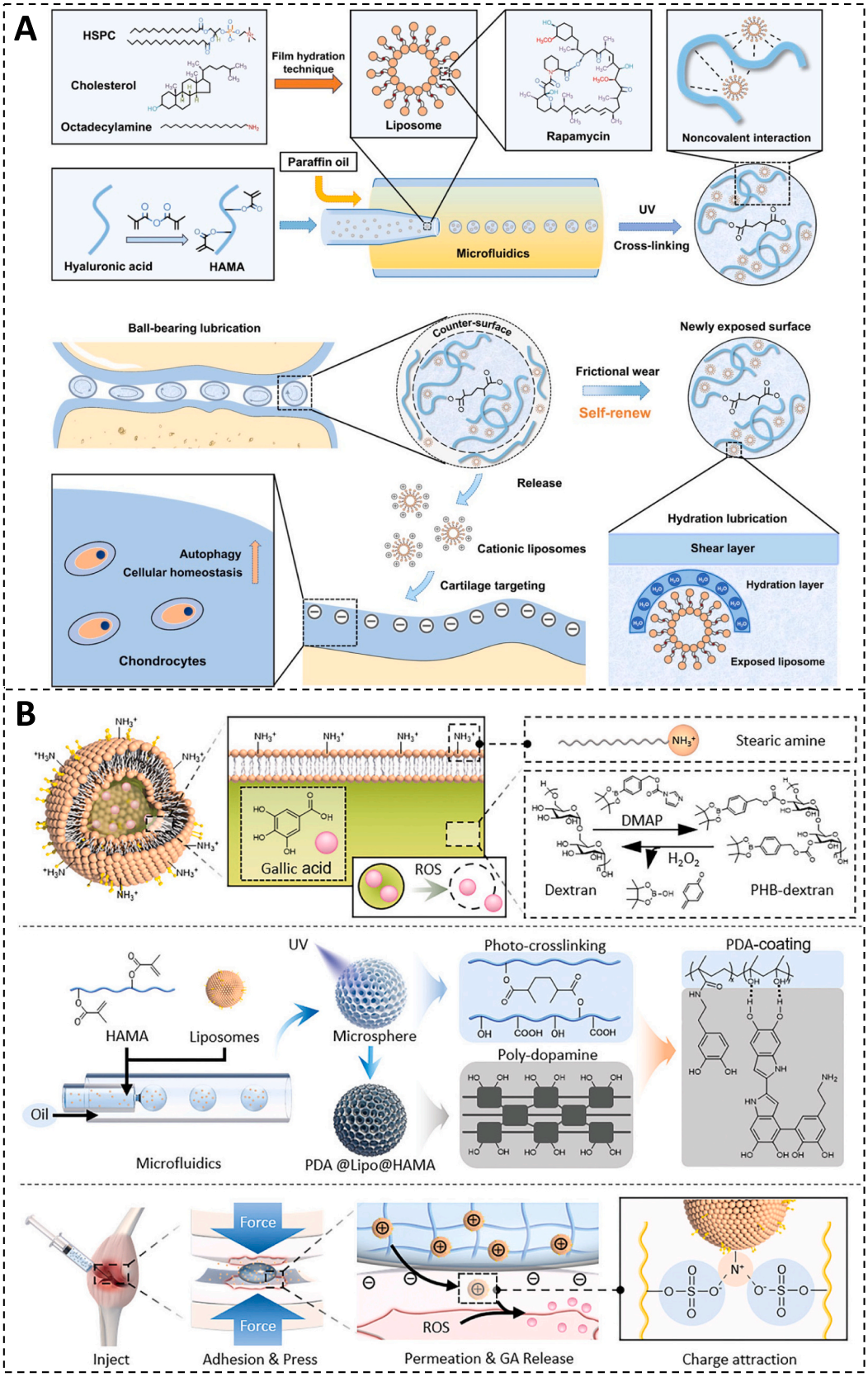

Fig. 14. Use of micro-/nanocarriers for the delivery of biologically active molecules for the repair of cartilage and suppression of OA. A) Design of Rapamycin loaded liposomes inside HAMA microspheres for treating osteoarthritis based on combining hydration lubrication and ball-bearing lubrication and maintaining cellular homeostasis. Reproduced from [281] under open access. B) Design of charge-guided micro-/nano-hydrogel microspheres for treating OA based on penetrating cartilage, ROS-responsive drug release and inhibiting chondrocyte apoptosis. Reproduced from [282] with permission from John Wiley and Sons.

Table 4
Injectable hydrogels that are commercially available and used clinically or injectable hydrogels that are currently undergoing trials.

Product Name	Trial(s) start - end	CE Mark	Company/Institute	Material and composition	Application	Clinical trial Identifier	Ref.
JointRep®	2021-2025	✓	Medicwave, (Malaysia)	Deacylated chitosan	Augmentation for microfracture	NCT04840147 (Recruiting)	[284–286]
CaRes®	2003-2005	✓	Arthro Kinetics (Esslingen, Germany)	Type I collagen from rat tail tendon with/ without autologous chondrocytes	Augmentation for ACI (MACI)	N/A	[287,288]
BST-CarGel®	2006-2015 2010-2018	✓	Smith & Nephew, (United Kingdom)	Chitosan solution buffer mixed with autologous whole blood before application after bone marrow stimulation technique	Augmentation for microfracture	NCT00314236 (Completed) NCT01246895 (Completed)	[289–291]
Hy2Care®	2022-2024	–	Hy2Care, (Netherlands)	Dextran and Hyaluronic acid conjugate	Cartilage defect repair for small lesions (0.5-2cm^2)	NCT05186935 (Recruiting)	[292,293]
Arthrosamid®	2022-2026 2021-2027	✓	Contura International Ltd., (Denmark)	2.5% cross-linked polyacrylamide and 97.5% non-pyrogenic water	Symptomatic treatment for knee OA	NCT05086068 (Not yet recruiting) NCT05057559 (recruiting)	[294,295]
Gelrin C™	2009-2019 2017-2023	✓	Regentis Biomaterials Ltd., (Israel)	PEG diacrylate and denatured fibrinogen (formed in situ via photopolymerisation)	Augmentation for microfracture	NCT00989794 (Unknown) NCT03262909 (Active, not recruiting)	[296,297]
NOVOCART® Inject plus	2015-2019 2017-2021	✓	Tissue Engineering Technologies AG (Germany)	Expanded autologous chondrocytes, modified human albumin, isotonic sodium hyaluronate, PEG crosslinker	Injection system for ACI procedure	NCT02941120 (Completed) NCT03319797 (Active, not recruiting)	[298]
ChondDux	2010-2017	–	Zimmer Biomet Holdings, Inc (USA, Indiana)	PEG/HA functionalised by CS (formed via photopolymerisation)	Augmentation for microfracture	NCT01110070 (Terminated)	[299–301]
CARTISTEM ®	2009-2011 2012-2021	✓	Medipost Co Ltd. (South Korea)	Hyaluronic Acid with allogenic human umbilical derived MSCs	Augmentation for microfracture	NCT01733186 (Completed) NCT01041001 (Completed)	[302,303]

may experience structural degradation, mechanical integrity loss, or excessive swelling over extended periods, hindering their capacity for sustained support and therapeutic delivery. Balancing biodegradation kinetics with tissue regeneration proves to be an ongoing challenge, where hydrogels that degrade too rapidly may impede the formation of new cartilage, while those that degrade too slowly may result in inadequate healing outcomes.

Successful cartilage repair also necessitates seamless integration of the hydrogel with the surrounding host tissue. Overcoming this hurdle of achieving strong bonding and seamless tissue integration remains a paramount objective, as poor integration can lead to fibrous tissue formation at the interface, limiting mechanical load transfer and reducing regenerative potential. Integration of the hydrogel will also allow for migration of endogenous chondrocytes from the native cartilage as well MSCs residing in the subchondral bone. Furthermore, strong adhesion of the hydrogel to the defect also aids the clinician since arthroscopic surgeries carried out under pressurised air or liquid could lead to dispersion of the hydrogel before crosslinking can begin to occur. Some recent advancements have explored incorporating specific moieties to enhance interaction with native cartilage [196,197], nonetheless further research is needed in this aspect.

Additionally, transitioning from laboratory-scale synthesis to large-scale production for clinical use poses practical challenges. Ensuring reproducibility, scalability, and batch-to-batch consistency of hydrogels are essential for regulatory approval and widespread adoption. The development of cost-effective manufacturing processes without introducing batch-to-batch variability is crucial in the translation of hydrogel-based therapies. Perhaps this challenge is one of the major reasons as to why there is still not as many hydrogels commercially available relative to the vast amount of research being done in this field.

Design parameters inherently differ based on whether the defect is focal or originates from degeneration due to osteoarthritis (OA). Osteoarthritic joints present a distinct microenvironment marked by factors such as a lower pH level and elevated matrix metalloproteinases (MMPs). These factors can expedite the degradation of scaffold materials when compared to implantation in focal defects [309]. In the case of focal defects, hydrogel design may emphasize controlled delivery of growth factors or chondroprotective agents to stimulate tissue regeneration. Conversely, defects resulting from OA may benefit from hydrogels engineered for the targeted release of anti-inflammatory drugs, given the inflammatory component associated with OA. It is essential to recognize that there is no one-size-fits-all solution in hydrogel design. Tailoring approaches to accommodate the unique needs of individual patients and specific joint conditions could be beneficial to improve patient outcomes. Customizing hydrogels to optimize the delivery of therapeutic agents, biomechanical properties, and other characteristics ensures a more precise and effective response to the cartilage defect location, origin, size and depth, microenvironment, amongst other patient requirements.

Nonetheless, the significant advancements in tissue engineering witnessed in recent years fuel optimism that these design challenges will be overcome, revolutionizing cartilage repair and ultimately improving patient outcomes. Continued interdisciplinary efforts and innovative approaches will pave the way towards realizing the full potential of injectable hydrogels for cartilage repair, offering a promising outlook for the field of regenerative medicine.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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