Biomaterials Science

ROYAL SOCIETY OF CHEMISTRY

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Biomater. Sci.*, 2025, **13**, 4916

From microparticles to bulk hydrogels: emerging granular hydrogels in cartilage tissue engineering

Akshat Joshi, *†[a,b,c] Akhilesh Agrawal,†[d] Saswat Choudhury,[e] Subha Narayana Rath,[f] Akshay Joshi,[g] Kushal Taori,[h] Savadamoorthi Kamatchi Subramani,[i] Sabari Murugesan,[i] Ujjayan Majumdar,[j] Ji-hoo Lee[a] and Suk-Jung Oh[a]

Articular cartilage exhibits a limited capacity for self-repair, prompting extensive research into advanced biomaterials that can support tissue regeneration. Among these, injectable hydrogels have gained attention for their minimally invasive delivery and suitability for bioprinting applications. However, conventional nanoporous bulk hydrogels often lack the necessary microporosity and architectural complexity to fully support effective tissue regeneration. To overcome these shortcomings, recent innovations have turned toward granular hydrogels—injectable materials fabricated by dense packing of hydrogel microparticles into cohesive, microporous bulk hydrogels. These granular systems offer improved injectability, superior microporosity, and the ability to form heterogeneous bioinks/injectables that better replicate the natural extracellular matrix, thereby promoting more efficient regeneration. This review delves into the advancements in granular hydrogel technology, with a focus on the fabrication of hydrogel microparticles and the jamming strategies used to assemble them into granular injectables/bioinks. It further explores their potential in cartilage tissue repair, emphasizing the benefits of such emerging microporous bulk assemblies in minimally invasive procedures (MIPs) or as smart bioinks for fabricating patient specific implants. Finally, the review outlines key opportunities and challenges in translating these innovative materials into clinical applications, highlighting the growing promise of granular hydrogels in addressing current limitations in cartilage regeneration.

Received 23rd May 2025,
Accepted 28th July 2025

DOI: 10.1039/d5bm00801h

rsc.li/biomaterials-science

1 Introduction

Cartilage tissue engineering remains one of the most demanding areas within biomaterials research, primarily due to the inherent difficulty of replicating the complex architecture and function of native cartilage.[1,2] Initial strategies in this field have largely focused on direct cell delivery and organoid-based approaches,[3] where chondrocytes or stem cells are introduced into the damaged area in hopes of promoting tissue regeneration.[4,5] While promising in theory, these methods have encountered several major hurdles in practice. Most notably, delivering cells at high densities—a necessity for effective cartilage regeneration—can inadvertently lead to undesired differentiation pathways.[6,7] Instead of forming hyaline cartilage, which is critical for smooth joint function, stem cells often take on osteogenic or fibroblastic fates, resulting in the formation of less durable fibrocartilage.[8,9] Moreover, organoid systems and spheroid-based methods, though they offer improved cell–cell communication compared to single-cell dispersions, often fail to generate the mechanical strength required for load-bearing applications. Without additional material support, these systems are generally too fragile for use in high-stress environments like articular cartilage.

In parallel, conventional tissue engineering approaches have relied heavily on bulk hydrogels composed of natural biomaterials[10–12] such as hyaluronic acid,[13] collagen,[14] silk

[a]*Department of Research and Development, EcoWorld Pharm Co., Ltd, South Korea. E-mail: akshat@ecoworldpharm.com*
[b]*Terasaki Institute for Biomedical Innovation, Los Angeles, CA 90024, USA*
[c]*Dr. D.Y. Patil Medical College, Hospital and Research Centre, Dr. D.Y. Patil Vidyapeeth, Pune, India*
[d]*Department of Bioengineering, Indian Institute of Science Bangalore, 560012, India*
[e]*Department of Materials Science & Engineering, Yonsei University, Seoul 03722, Korea*
[f]*Regenerative Medicine and Stem Cell (RMS) Lab, Department of Biomedical Engineering, Indian Institute of Technology, Hyderabad, Telangana, India*
[g]*Department of Pharmacology and Regenerative Medicine; Department of Biomedical Engineering, University of Illinois at Chicago, Chicago, IL, 60612, USA*
[h]*Department of Orthodontics and Dentofacial Orthopedics, Sharad Pawar Dental College, Datta Meghe Institute of Higher Education and Research (DU), Wardha, 442001, India*
[i]*Department of Restorative Dental Sciences, College of Dentistry, Jazan University, Jazan, Saudi Arabia*
[j]*Department of Biomedical Engineering, Columbia University, New York, NY 10027, USA*
†Equal contribution.

fibroin,[15] and chitosan[16] among widely utilized hydrogel systems. These hydrogels offer biocompatibility and support cell viability, and they can mimic some aspects of the extracellular matrix. However, they frequently fall short when it comes to recapitulating the densely packed, organized structure of native cartilage. A major limitation lies in the low cell density typically used within bulk hydrogels, which impairs cell–cell interactions that are critical for maintaining chondrocyte phenotype and promoting the expression of cartilage-specific markers. Furthermore, culturing chondrocytes on 2D plastic surfaces—still a standard step in many hydrogel-based strategies—tends to induce de-differentiation, turning the cells into fibroblast-like cells that lack the ability to form true hyaline cartilage. As a result, these bulk hydrogel systems often fail to replicate the mechanical robustness and biological fidelity needed for functional cartilage repair. Despite recent advancements leading to sophisticated versions of bulk hydrogel systems,[17] their full potential remains limited due to persistent challenges such as poor conformability, limited adaptability, lack of structural heterogeneity, and insufficient nanoporosity.

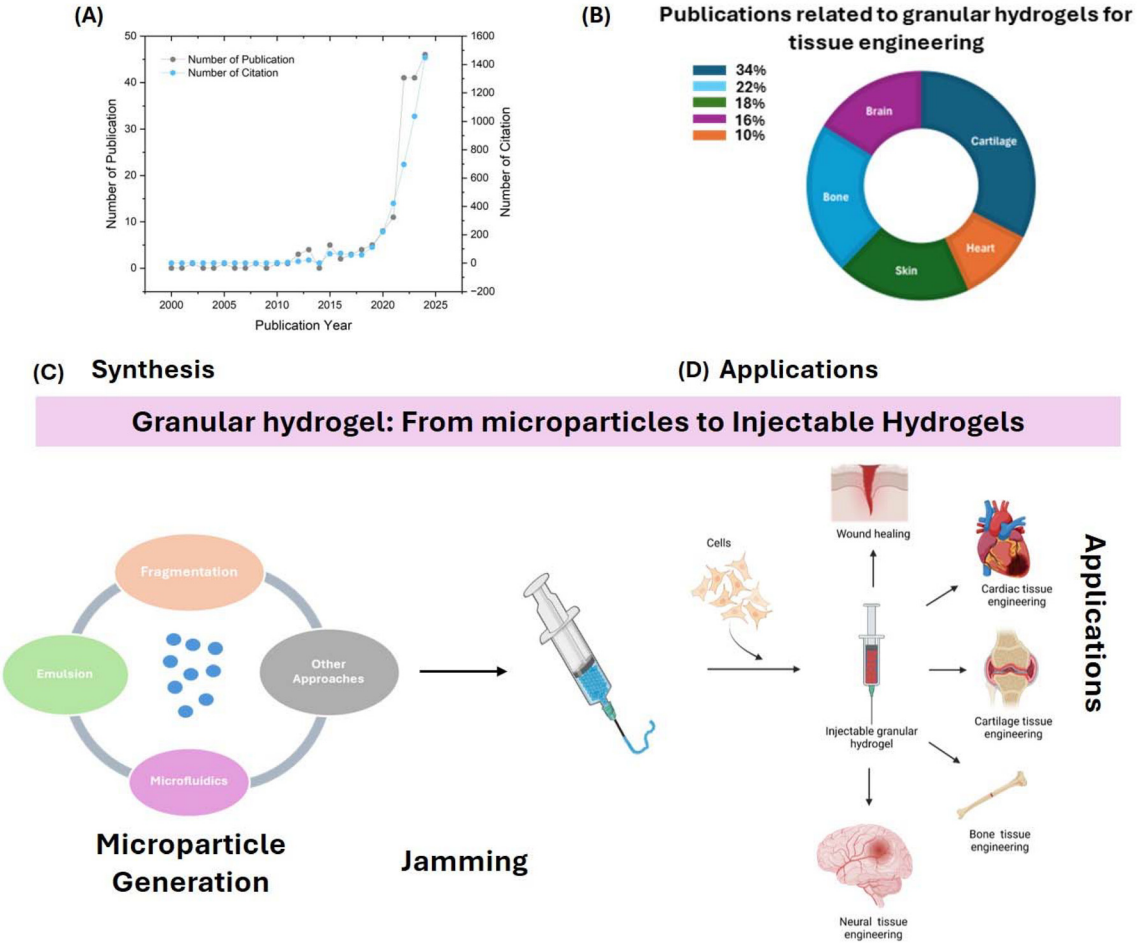
In response to these limitations, granular hydrogels have emerged as a next-generation material platform with significant promise for cartilage tissue engineering.[18] These hydrogels are created by jamming together hydrogel microparticles into a porous, yet cohesive, structure. This granular architecture offers several compelling advantages. For one, the jammed microgel environment allows for high cell densities while simultaneously facilitating enhanced cell–cell contact and communication. This mimics the cellular organization found in native cartilage and promotes the appropriate differentiation of stem cells into chondrocytes, avoiding the pitfalls seen in bulk hydrogels or cell-only approaches.[19] Additionally, the mechanical stability of granular hydrogels is substantially improved, making them more suitable for load-bearing applications. They also offer a unique advantage in terms of versatility and application: their injectability and printability enable the creation of patient-specific constructs[20,21] and even envisions minimally invasive delivery through robotic surgical techniques.

Given these favorable characteristics, granular hydrogels have garnered significant interest in recent years for use not only in cartilage repair but also in the regeneration of other complex tissue types (Scheme 1). The current review aims to provide a thorough overview of granular hydrogel fabrication methods, particularly the strategies used to induce jamming and build stable constructs. Special attention is given to their application in cartilage tissue engineering, including their translational potential and the remaining scientific and clinical challenges that must be addressed to bring these materials from the research lab to clinical use. By highlighting the shortcomings of current regenerative therapies and underscoring the transformative features of granular hydrogels, this review supports their development as a minimally invasive, clinically relevant solution for effective cartilage repair and regeneration.

1.1 Structural characteristics of cartilage, pathogenesis, and current tissue engineering strategies

1.1.1 Structure of cartilage. Articular cartilage is a hyaline cartilage found between the bones that acts as a load-bearing site and helps in lubrication to provide a cushioning effect during movements. Unlike most tissues, cartilage is devoid of vascular, lymphatic, and nerve supply.[22] Hence, it relies on the diffusion of nutrients from the surrounding tissue. The heterogeneity and anisotropy of this stratified tissue are always depth-dependent and are categorized into four different zones.[23,24] The zones are superficial, middle, deep, and calcified, characterized by variations in collagen fibers and ECM content alignment. The superficial zone is a densely packed region with collagen fibers parallel to the cartilage surface, occupying 10–20% of the complete cartilage. The zone has the lowest glycosaminoglycan (GAG) level, and the chondrocytes here are flat, compact, and densely packed.[25] The middle zone comprises 40–60% of the cartilage and is distinguished by randomly arranged collagen fibers. It has a high GAG content, and chondrocytes are distributed randomly across the collagen fibers. The deep zone holds 30–40% of the cartilage thickness, characteristic of radially arranged collagen fibers and chondrocytes. This zone has the highest GAG content.[24,26] The calcified zone is adjacent to the subchondral bone, in which the collagen fibers arborize with little organization and mineralization.[27] This stratified structural arrangement endows unique properties and functions to each layer of the cartilage (Scheme 2). The superficial region possesses excellent tensile strength due to collagen fibers. Further, a transition towards increasing compressive strength is observed towards deeper zones due to increasing GAG content.[28] These physicochemical properties of the cartilage result from highly enriched ECM, which contains cartilage collagen, hyaluronic acid, proteoglycans, and water.

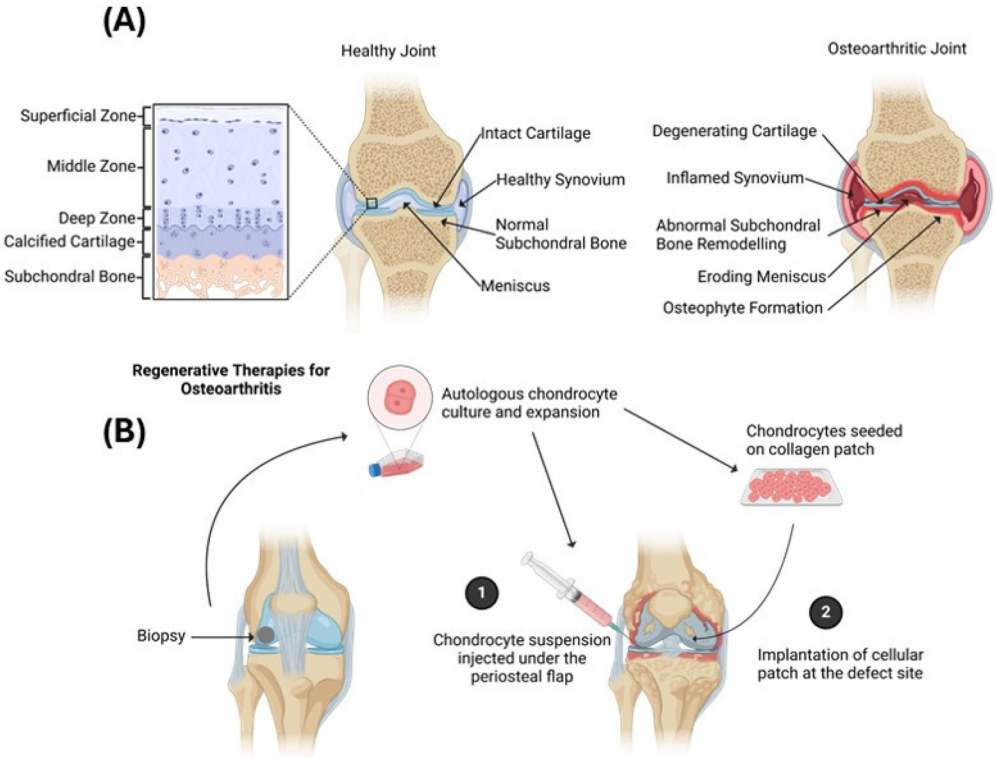
1.1.2 Overview of cartilage pathogenesis. Due to the load-bearing function, cartilage tissue is susceptible to injury and degeneration owing to extensive physical activity or trauma.[29] Osteoarthritis is a major cause of cartilage pathology arising from the disturbed homeostasis between the cells and the ECM. The catabolic activities surpass the anabolic activities, further deteriorating cartilage health upon biomechanical loading.[30,31] During the initial phase of osteoarthritis, the chondrocytes become metabolically hyperactive, and the ECM is swollen due to excessive hydration.[32] This leads to the development of fissures on the cartilage surface. Concomitantly, remodeling changes are also seen in the subchondral bone. During the progression of the pathogenesis, the loss of proteoglycans results in the disruption of the collagen network, and delamination of the cartilage occurs, exposing the deeper zones of the tissue and the bone (Scheme 2).[33] The inflammatory load orchestrates the disease severity in the synovial cavity, and the cytokines are the main contributors to the progression of osteoarthritis.[34] Depending on the depth involved, the defects are categorized as: i: partial thickness chondral defect, ii: full-thickness chondral defect, and iii: osteochondral



Scheme 1 Emerging trends of granular hydrogels for biomedical application. (A) Exponential growth in the research of granular hydrogels. The data is obtained from the ISI Web of Science using keywords, "granular hydrogel" OR "granular polymers". (B) Publications related to granular hydrogels in various biomedical fields. Data obtained from ISI Web of Science using "granular hydrogel or granular polymer" AND "skin OR heart OR cartilage OR bone OR brain" (March 2025). (C and D) Fabrication process and potential utilization of granular hydrogels as injectable biomaterial in various field of tissue engineering. Created with BioRender.com.

defect (involves subchondral bone).[35] The success of clinically available regenerative treatments is greatly affected by the extent of the defect involved, and in many cases, it is very challenging to achieve good clinical outcomes.

1.1.3 Challenges in current cartilage tissue engineering strategies. As discussed above, pathological conditions such as osteoarthritis resulting in cartilage injury and degeneration often lead to chronic disability in otherwise healthy populations. The extent of cartilage tissue involved in the pathogenesis can significantly affect the repair mechanisms and pose a major clinical challenge. Furthermore, cartilage lacks inherent vascular and lymphatic supply and progenitor cells. The scarcity of chondrocytes and their localization in the superficial and deep zones also limits the repair of cartilage injury. The clinically available regenerative modalities for cartilage tissues still remain ineffective as they require an extensive duration for healing, which relies on the integrity and support of the underlying bone.[36,37] The modern cartilage regenerative therapies, such as autologous chondrocyte implantation (ACI) and matrix-associated ACI (MACI), face clinical challenges as these treatments face to recapitulate long-term cartilage healing, donor site morbidity, multiple surgeries, tissue loss, *etc.*[1] The viscosupplementation treatment, which includes injecting hyaluronic acid into the joints to alleviate pain and inflammation, requires multiple injections as the hyaluronic acid is not retained at the site for a long duration.[38] Therefore, there is a primary need for newer and more effective cartilage regeneration therapies. Cartilage tissue engineering using biomaterials has been significantly explored and holds promise to provide better healing strategies for cartilage. Specifically, injectable hydrogels have gained a lot of attention as they can be delivered in a minimally invasive manner and/or can be 3D bioprinted to develop patient specific implants, that can easily fit defects.[29] Granular hydrogels, which are microporous versions of traditional bulk hydrogels have evolved as emerging alternatives due to their superior and tuneable properties such as microporous structure, pores interconnectivity, injectability, and self-healing properties. Cell-to-cell communications can



Scheme 2 Overview of cartilage physiology, pathogenesis, and tissue engineering approaches. (A) Schematic illustration of the structural arrangement of articular cartilage and degenerating changes occurring during cartilage pathogenesis, like in the case of osteoarthritis. (B) Current regenerative therapies as a treatment modality for osteoarthritis. Created with BioRender.com.

be easily achieved through granular hydrogels, thereby accelerating chondrogenesis.[39] Thus, the building evidence suggests that granular hydrogels can be very useful in the repair and regeneration of complex biomimetic tissues like cartilage.

1.1.4 Minimally invasive strategies for cartilage tissue engineering. The existing treatment modalities for cartilage treatment do not demonstrate long-term efficacy, and newer tissue engineering approaches are needed to address the unmet clinical needs. Injectable hydrogels have shown tremendous improvement in cartilage regeneration administered *via* minimally invasive procedures (MIPs). MIPs remains the first clinical choice of treatment for patients who cannot withstand invasive surgical procedures like osteoarthritis surgical treatments. Biomaterial delivery using this approach has several advantages, like minimal blood loss, rapid recovery, short procedure time, and less trauma.[40,41] Injectable hydrogels have a certain viscosity or fluidity, allowing their injection at the desired site and the *in situ* gelling properties to form a 3D network for cellular repair. The use of injectable hydrogels for drug delivery and tissue engineering applications, including cartilage tissue engineering hydrogels, have been exclusively studied due to features like biocompatibility, tuneable physical and mechanical properties, ECM mimicking ability, ease of handling, and minimally invasive delivery.[42] Recent studies have constituted evidence about the limitations of such bulk and traditional hydrogels. They introduce barriers to cellular infiltration and tissue ingrowth that can diminish the hydrogel integrity with the host tissues. This is due to the covalently bonded polymer chains resulting in the nanoporous hydrogel.[43] Upon loading the cells with the bulk hydrogels, they tend to agglomerate due to low cell-to-cell communication, which can impede their function, particularly for cartilage regeneration.[44,45] The encapsulated cells further cannot survive for a long duration due to the scarcity of diffused nutrients. The cell-free approach cannot recruit cells and allow their infiltration for tissue regeneration. Considering these aspects, though traditional hydrogels are biocompatible with tuneable biodegradability and can be delivered through a minimally invasive approach, the aforementioned limitations can be a bottleneck for the clinical translation of bulk hydrogels in the clinics.[46,47] Another class of injectable hydrogels, granular porous hydrogels, have emerged as a new biomaterial-based tissue regenerative strategy to address the limitations of bulk hydrogels. Granular hydrogels with microporosities ranging between 10–300 μm offers several advantages, allowing them to be delivered using MIPs.[48] Cell-laden granular hydrogels can facilitate cell–cell contact, which enhances chondrogenesis, an efficient supply of nutrients, and the clearance of wastes, leading to cell proliferation, migration, and differentiation. The micro-scale pores formed by the granular hydrogel enable tissue ingrowth, ensuring proper integration with the host tissues. In addition, the biodegradation, mechanical, and

biological properties can be significantly tuned as per the host tissue by tuning properties of microgels.[49–52]

1.1.5 3D bioprinting approaches for cartilage tissue engineering. The extent of cartilage involved in osteoarthritis varies depending on the disease state and progression. The cartilage defects are divided into partial-thickness and full-thickness defects. It would be more beneficial to design the treatment strategy for cartilage defects according to the defect size and shape.[22,53] The technical difficulty in regenerating full-thickness cartilage defects is to simulate the structural, mechanical, and biological properties, which are different in each phase (layer) of cartilage.[54] Utilizing 3D (bio)printing technology to fabricate mechanically resilient and sufficiently larger scaffolds for full-thickness cartilage defects would be more advantageous and clinically relevant. This section highlights the potential of 3D bioprinting techniques to fabricate tissue-specific scaffolds with outstanding structural precision for complex cartilage defects.

3D bioprinting utilises cell-biomaterial composite-based bioinks to develop scaffolds with remarkable biomimicry. The technology enables controlled deposition of cell-laden hydrogels in a desired fashion to achieve morphological and cellular gradients present in natural cartilage tissue.[55] The current 3D bioprinting approaches that can be leveraged to fabricate tissue prototypes include extrusion-based, droplet-based 3D printing, and light-based approaches such as Digital Light Processing (DLP), Stereolithography (SLA), two photon polymerization, and laser-induced forward transfer bioprinting. Among these, extrusion-based bioprinting is a widely preferred technique as it offers the ease of using a variety of biomaterials and cell types and enables 3D printing of complex geometries.[56] During extrusion, the hydrogel bioink must undergo a viscoelastic transition where it assumes a fluidic state upon applied pressure so that it extrudes as a continuous filament and solidifies after deposition on the printing bed. For extrusion-based bioprinting, this balance between the ink solidification and deposition rate is crucial to fabricate tissue-mimetic scaffolds.[57,58] However, the traditional bioinks prepared from bulk hydrogels pose significant challenges during 3D bioprinting procedures, impeding their translation into the clinics. For example, a highly viscous bioinks prepared from dense polymer concentrations provide optimal printability, shape fidelity, and stability of the constructs. But it fails to prevent cellular damage caused by shear stress during extrusion, and it further inhibits cell growth and differentiation due to a sub-optimal microenvironment. Another challenge is to bioprint a tissue construct of a desired height due to uneven hydrogel crosslinking and their mechanical insufficiency.[59] Also, traditional bioinks show a decrease in overall porosity and pore interconnectivity of the scaffolds, which will eventually compromise the cellular and biological functions of the scaffolds.[60,61]

Due to their dynamic rheological behaviour, granular hydrogels are emerging as potential alternatives for extrusion-based 3D bioprinting compared to traditional hydrogels. As granular hydrogels show excellent structural, shear-thinning, and self-healing properties, they extraordinarily exhibit a transition from solid to liquid state upon applied stress and thus are the most suitable bioinks for extrusion-based 3D bioprinting.[62,63] The exceptional rheological properties of the granular hydrogels may establish a new paradigm for the bioprinting of a set of polymers and cells, which are challenging to print using traditional bioprinting methods for cartilage regeneration. Another distinct advantage of granular hydrogel bioinks over traditional bioinks is the ability to tune the scaffold porosities by manipulating the size and shape of the hydrogel microparticles. This feature can affect the cell migration and alignment drastically, along with optimal cell–cell interactions, which are desired for chondrogenesis. This unique class of granular hydrogel-based bioinks offers improved biofabricated structures to recapitulate tissue-mimicking architecture for enhanced cellular functions and integration with the host tissues.

2 Granular hydrogels: An overview of microparticle design strategies and jamming mechanisms

2.1 Granular hydrogel *vs.* bulk hydrogels

Articular cartilage regeneration heavily depends on the ability of hydrogels to facilitate efficient nutrient diffusion and cell survival for both encapsulated and migrated cells. Bulk nanoporous hydrogels, however, often struggle to provide sufficient microporosities and pore interconnectivity necessary for efficient diffusion and nutrient exchange. These limitations can result in low cell survival rates and hinder the migration of surrounding cells, which is crucial for effective tissue regeneration. Also, when bulk hydrogels are utilized for developing patient specific implants using 3D bioprinting approaches, their nano porosities hinder efficient maturation of cells due to diffusion limitations. In contrast, granular hydrogels, with their enhanced microporosity, offer significant advantages in supporting cell survival, migration, and the development of neo-vasculature, which is essential for long-term tissue regeneration.

Granular hydrogels not only provide better pore connectivity but also support more effective cell recruitment. Their increased microporosity creates a conducive environment for the migration of cells into the hydrogel matrix, aiding in the formation of new tissue. This property is particularly beneficial in cartilage tissue engineering, where the ability to recruit host cells and support blood vessel formation is crucial for repairing damaged cartilage. Moreover, granular hydrogels exhibit increased potential as injectable materials, enabling their delivery *via* minimally invasive procedures, which is a significant advantage in clinical applications.

One of the key benefits of granular hydrogels lies in their injectability. Their granular structure allows them to be easily injected into the site of injury or degeneration, where they can expand and integrate with the surrounding tissue, forming a

stable scaffold for tissue regeneration. This injectability not only minimizes the need for invasive surgery but also allows for precise targeting of the damaged tissue, reducing recovery times and complications associated with traditional surgical approaches. This feature makes granular hydrogels particularly attractive for treating cartilage defects, where minimizing the invasiveness of treatment is essential for patient recovery.

In addition to injectability, granular hydrogels offer excellent printability for developing *in situ* cartilage implants. By leveraging advances in 3D printing technology, granular hydrogels can be precisely printed to create customized, patient-specific cartilage implants. The design flexibility of granular hydrogels, combined with their structural stability, makes them ideal candidates for 3D printing, allowing for the development of implants that can be tailored to the exact shape and size of the cartilage defect. This capability ensures a better fit and more effective integration of the implant with the surrounding tissue, leading to improved outcomes in cartilage repair and regeneration.

Furthermore, the regenerative potential of granular hydrogels can be enhanced by controlling microgel design parameters such as particle size, shape, and mechanical properties. These parameters can be optimized to tailor the hydrogel's behavior to specific tissue engineering needs. The jamming behavior of the hydrogel particles can also be finely tuned to improve the mechanical properties, providing additional support to the regenerating tissue while maintaining the hydrogel's injectability and printability. By manipulating these factors, granular hydrogels offer a high degree of control over the scaffold's performance, which is crucial for the success of cartilage tissue engineering.

In summary, granular hydrogels offer several advantages over bulk hydrogels in the context of cartilage tissue engineering. Their superior microporosity, ability to support cell migration and neo-vasculature development, and potential for injectability make them highly suitable for minimally invasive treatments. Additionally, their printability allows for the creation of customized, *in situ* cartilage implants, further enhancing their clinical applicability. The ongoing exploration of granular hydrogels holds great promise for advancing the field of cartilage regeneration, with the potential for developing clinically translatable injectable materials that can provide more effective, personalized treatments for cartilage defects. Table 1 below summarizes the potential advantages of granular hydrogels over bulk hydrogels, necessitating their exploration in cartilage tissue engineering.

2.2 Hydrogel microparticles design considerations for cartilage tissue engineering

Recent studies on utilization of granular hydrogels for biomedical applications indicate a plethora of advantages over bulk hydrogel owing to its microporous nature. The choice of biomaterial for synthesizing microgels with specific design characteristics and functions is very complex, and it needs meticulous consideration of various factors. An appropriate microgel component that can positively manipulate granular hydrogel's mechanical and biological properties can effectively orchestrate cartilage regeneration.[11] Microscale interconnective pores are important for the efficient flow of nutrients, waste, and tissue formation. The granular hydrogel shows abundant voids and interconnected micropores, which can be tuned by tailoring the size and shape of microgels to promote chondrocyte adaptation.[47,64] Compared with bulk hydrogels, which consist of densely packed polymer chains, the interconnected microscale void spaces can facilitate the diffusion and flow of nutrients and signaling molecules for cellular functions.[65] The void spaces also provide the passage for cell and tissue infiltration, which in other cases is only possible after hydrogel degradation.[66] The elastic nature of articular cartilage provides smooth and frictionless movement between the bones and helps distribute the forces due to its load-bearing and lubrication properties.[67] The microgels should be mechanically sound to withstand the load and resist deformation under stress to match the native cartilage.[68] The bulk hydrogels impose challenges during injection at the defect site. In most scenarios, the precursor hydrogel solutions are injected which exhibit liquid-like rheological properties, and then subsequently crosslinked to form a gel. This approach can either result in an incomplete crosslink (inefficient penetration depth of the light for photo crosslinker) or can

Table 1 Advantages of granular hydrogels over bulk hydrogels for cartilage tissue engineering

Hydrogel parameters for cartilage tissue engineering	Granular bulk hydrogel	Traditional bulk hydrogel
Porosity control	Easily achievable by controlling micro-particle shape, size, and jamming mechanisms	Controlling porosities is difficult to achieve. Often nanoporous compromising their performance
Cell Infiltration	Microporosities allow efficient cell infiltration	Nano porosities hinder cell infiltration
Control on angiogenesis	Controlled angiogenesis prevents hypertrophy	Limited control often leads to fibrotic capsules development
Heterogeneity	Easily achievable through variation in microgel properties	Difficult to achieve
As injectables for MIPs	Good shear thinning properties allowing easy injections with low shear induced damage to loaded cells	High extrusion forces might lead to compromised cellular viability
As bioinks for 3D bioprinting	Shear thinning properties allows efficient printability with microporosities enhancing tissue maturation	Poor printability, often requires addition of shear thickeners. Further, diffusion limitations in bioprinted constructs hinders tissue maturation.

damage the host tissues while crosslinking it using chemicals.[69] In contrast, granular hydrogels developed from cross-linked hydrogel microparticles offers improved injectability and enhanced flowability allowing easy injections at the desired location. The reduced shear stress in the granular hydrogels during injection can increase cell viability. Introducing dynamic bonds within the microgel can endow good stability and self-healing properties post-extrusion from the syringe.[70] Most tissue regeneration applications, including cartilage regeneration, require the implanted material to be degraded once the function is achieved with minimal or no cytotoxicity. An implanted biomaterial can undergo various mechanisms of biodegradation, such as hydrolytic cleavage, enzymatic attack, oxidation and reduction reactions, *etc.*[71,72] The degradation profile of the microgel will be dependent on the chemical structure, degree of interparticle crosslinking, and swelling capacity of the microgel, as it will impact the diffusion kinetics of molecules and enzymes. Therefore, an appropriate choice of biomaterial and crosslinking mechanisms should be employed to program the degradation rate of microgels for a particular application. Many of the granular hydrogel systems for cartilage tissue engineering have been loaded with cells, including stem cells and chondrocytes. Encapsulating cells in the granular hydrogels can protect them from oxidative stress and increase long-term efficiency. Thus, microgel components mimicking extracellular matrix can promote cell viability, adhesion, and proliferation. RGD peptides can be introduced in microgels made from biomaterials that do not support cellular adhesions.[73–75]

2.2.1 Fabricating hydrogel micro particles. Granular hydrogels are developed by compacting hydrogel particles into tightly packed assemblies, which are distinguished by their high extrudability and the presence of micro-porous structures.[18,76] These densely packed assemblies can be injected in a minimally invasive manner or can be utilized as bioinks, offering a promising approach for the repair of damaged tissues or defects. Compared to their bulk counterparts, the presence of microporosities in granular hydrogel can enhance their performance by allowing effective infiltration of host cells, altering macrophage phenotype, allowing effective vascularization deep into constructs, and ensuring long-term survival of loaded/migratory cells at the site of injury.[77] Over the years, various strategies have been developed to fabricate hydrogel particles that can be subsequently jammed into granular hydrogels. These strategies encompass a broad range of techniques, each of which comes with their unique advantages in terms of their production rate, scalability, and ability to generate micro-particles of different shapes and dimensions, which can greatly impact the performance of granular hydrogels. Herein, a brief overview of techniques utilized to produce micro-particles from bulk hydrogel materials, along with advantages and challenges of each technique are discussed with insights on future possibilities.

2.2.1.1 Mechanical fragmentation. Fragmentation is a widely utilized technique for generating microparticles from bulk hydrogels, particularly favoured for its simplicity and efficiency.[78,79] This process involves the mechanical force applied to crosslinked bulk hydrogels, which are pushed through sieves or needles, or simply blended breaking them into microparticles.[80] The size of these microparticles can be meticulously controlled by adjusting the pore size of the sieve or the diameter of the needle or by controlling the blending speed/time, with typical particle sizes ranging up to several hundred microns. Once formed, the microparticles are usually suspended in physiologically compatible solvents, such as cell culture media or saline, to facilitate their collection and subsequent separation.[81] Techniques like filtration or centrifugation are then employed to isolate the microparticles from the surrounding solvent, ensuring that they are recovered intact and ready for use. These microparticles can then be jammed through various mechanisms into granular hydrogels that are highly injectable and can be utilized for various applications in biomedicine, including tissue repair,[82] drug delivery,[83] and as bioinks for 3D bioprinting (Fig. 1A).[84,85]

Fragmentation technique allows fabrication of injectable granular hydrogels from their non-extrudable bulk counterparts, extending their application for minimally invasive therapeutic applications.[86] For instance, zwitterionic hydrogels, known for their excellent non-immunogenic[87] and anti-fouling properties,[88] are especially valuable for injectable therapies aimed at treating cartilage injuries. However, these hydrogels often suffer from poor extrudability, which limits their clinical applicability. By applying extrusion fragmentation, the bulk hydrogels can be transformed into granular forms, significantly improving their extrudability and enabling their use in cartilage tissue engineering (Fig. 1B).[86] This approach has also been applied to alginate-based hydrogels, which, when fragmented into granular forms, exhibit enhanced thixotropic properties, improved extrudability, and increased chondrogenic potential (Fig. 1C).[89,90] While bulk alginates lack the desirable injectable properties for clinical use, their granular variants address this challenge, offering enhanced mechanical and biological performance.

Despite its many advantages, extrusion fragmentation is not without its limitations. One key issue is the generation of microparticles with irregular shapes and inconsistent sizes, often resulting from the high shear forces required to break down the bulk hydrogels. This lack of uniformity can complicate the process, especially when aiming for particle sizes consistently below 100 microns, which can lead to issues like nozzle blockage during injection. To achieve more homogeneous microparticles, additional filtration steps may be needed, introducing extra complexity and time into the process. Moreover, the technique lacks full automation and provides limited control over the fine-tuning of microparticle properties, which can constrain its scalability and precision.

Nonetheless, extrusion fragmentation remains an invaluable technique for bridging the gap between laboratory research and clinical application. Its simplicity, cost-effectiveness, and ability to operate without the need for complex setups or organic solvents make it a promising approach for high-throughput production of injectable hydrogels. Moreover, non-spherical flaky particles generated using this technique

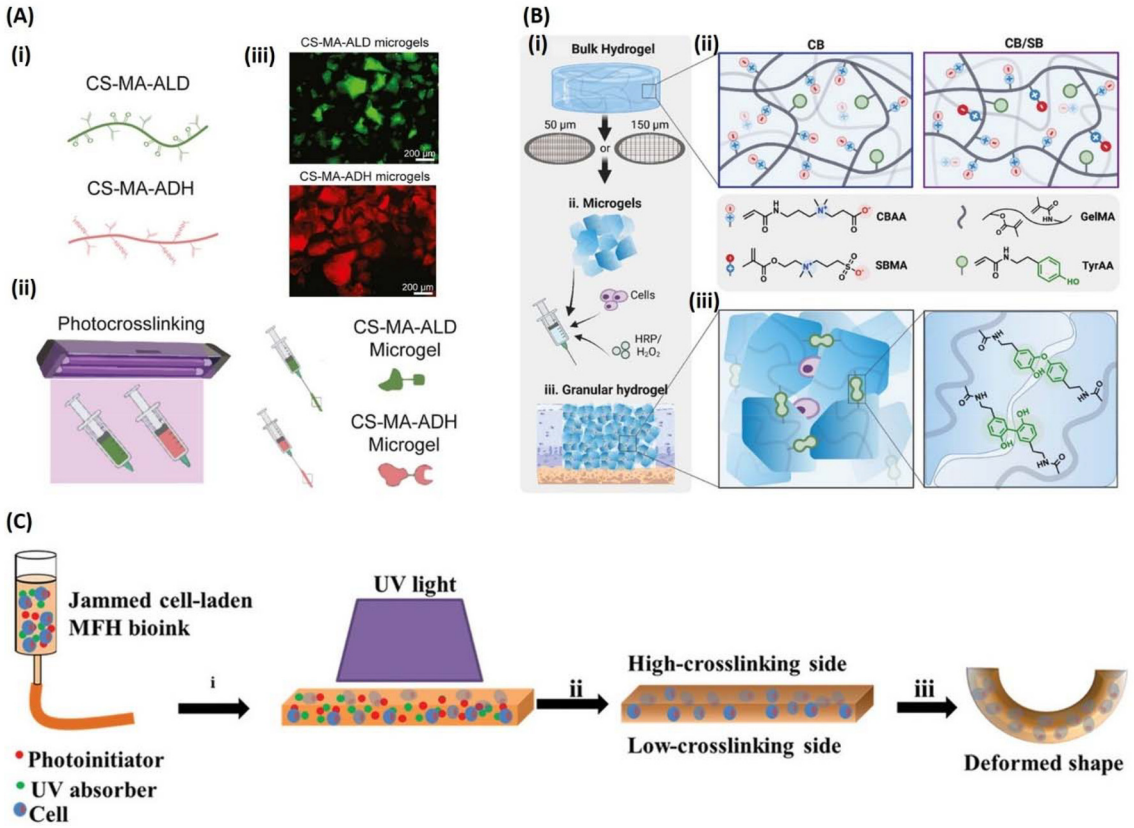


Fig. 1 Utilization of mechanical fragmentation approach for creating hydrogel microparticles for cartilage tissue engineering. (A) Dynamic granular hydrogel based on hydrazone chemistry can be developed from chondroitin sulfate microparticles fabricated using mechanical fragmentation. (i) Schematic illustration demonstrating functional modification of chondroitin sulfate methacrylate (CSMA) with aldehyde and hydrazide groups that can be utilized for annealing of fragmented microgel particles using hydrazone chemistry, (ii) the process of mechanical fragmentation wherein the bulk hydrogels from CSMA-ALD or CSMA-ADH are first formed using photo crosslinking of precursors within the cartridge and the subsequently fragmented using different needle gauge to obtain microparticles, (iii) microscopic images of generated particles post fragmentation, reproduced from ref. 85 with permission. Copyright©2023, American Chemical Society. (B) Zwitterionic bulk hydrogels can be fragmented to generate microparticles using mechanical fragmentation. (i) Schematic representation demonstrating generation of microparticles from bulk hydrogels that can be mixed with cells (chondrocytes or stem cells) and annealed into granular hydrogels using enzymatic driven jamming which can be then utilized as injectables or as bioinks, (ii) bulk hydrogel structure made of either pure carboxybetaine acrylamide (CB) or a mixture of CB and sulfobetaine methacrylate (SB) crosslinked with GelMA incorporating functional comonomer, TyrAA that can be utilized for annealing of microparticles, (iii) schematic illustration demonstrating mechanism of particle jamming to obtain granular hydrogels from fragmented zwitterionic microparticles, reproduced from ref. 86 with permission. Copyright©2024, Wiley-VCH GmbH. (C) Jammed alginate based granular hydrogel bioink developed using extrusion fragmentation approach can be utilized for 3D/4D bioprinting applications, reproduced from ref. 90 with permission. Copyright©2022, Wiley-VCH GmbH.

provides larger surface area for jamming, ensuring efficient and stable granular injectables. Despite its current challenges, the continued development of this method holds great potential for advancing biomedical applications, particularly in the field of regenerative medicine, where the demand for versatile, injectable hydrogel-based systems continues to grow.

2.2.1.2 Emulsification. Emulsification represents a well-established and widely employed bottom-up technique for the fabrication of microparticles from bulk hydrogel precursors.[91,92] This approach generally involves two immiscible phases: a dispersion phase, typically composed of oil and surfactants, and a dispersed phase containing hydrogel precursors that include photo-initiators or crosslinkers. The two phases are mixed in specific ratios, with the oil phase usually present in volumes 5 to 10 times greater than the hydrogel phase.[93–95] Through agitation, this results in the formation of droplets of the hydrogel precursor. These droplets are subsequently crosslinked to form microparticles. The hydrogel microparticles are then separated from the oil phase *via* centrifugation, followed by washing with solvents, such as PBS or cell culture media, to remove residual oil. These microparticles can be used immediately, especially when cell-loaded for applications such as cell encapsulation, or they can be stored for later use. The size of the droplets can be easily controlled by adjusting several parameters, including the agitation speed, the ratio of oil to hydrogel phases, the concentration of surfactants, as well as factors like reaction temperature and mixing duration. Emulsification is particularly advantageous in creat-

ing microparticles of uniform size, typically in the micron range (50 microns), in contrast to extrusion-based fragmentation methods, where particle size is more difficult to control, allowing fabrication of injectable granular assemblies that can be utilized for minimally invasive cartilage regeneration (Fig. 2A).[96] This uniformity is key in ensuring consistent properties across fabricated granular hydrogels.[97]

Compared to granular bioinks made by fragmentation methods, which often result in irregularly sized and larger particles, emulsification enhances the injectability and regenerative performance of the resulting granular hydrogels.[98] For example, emulsions have been utilized to generate microgels from hydrogel precursors such as oxidized alginate or hydrazide-modified gelatin methacryloyl (GelMA),[84] which exhibit excellent injectability and self-healing properties. By exploiting hydrazone bonds, these microparticles can be fused to form granular bioinks, suitable for 3D or 4D bioprinting applications.[84] Similarly, thiol–ene chemistry has been used to

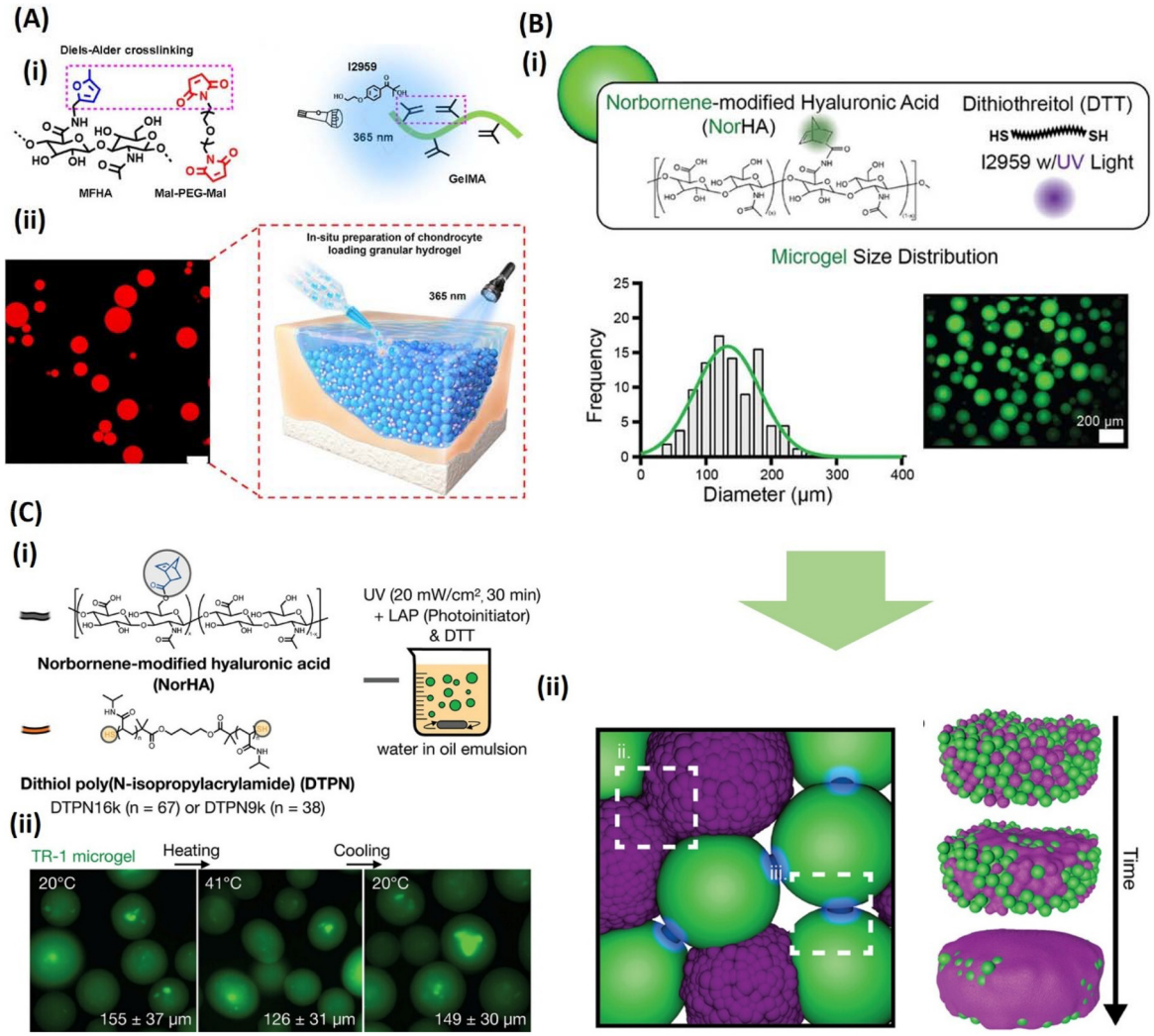


Fig. 2 Emulsification approach for fabricating hydrogel micro-particles and subsequent granular assemblies for cartilage tissue engineering. (A) Injectable granular hydrogels can be developed utilizing photo-annealable microgels fabricated using the emulsion technique. (i) Emulsion-based micro-particle fabrication, wherein Diels–Alder crosslinking was used to allow micro-particle fabrication using water in oil emulsion, (ii) fabricated microgels can be mixed with chondrocytes and annealed into injectable granular assemblies using light, which can be utilized for minimally invasive cartilage treatments, reproduced from ref. 96 with permission. Copyright©2022, American Chemical Society. (B) Granular composites developed using norbornene modified hyaluronic acid microparticles fabricated using the emulsion technique and combined with MSCs spheroid can mature into fully functional cartilage tissue *in situ*. (i) Photo clickable Nor-HA microparticle generation using emulsion with particle generation in the range of 100–200 microns, (ii) developed microparticles can be combined with MSCs spheroid to injectable granular composite with enhanced chondrogenesis, reproduced from ref. 99 with permission. Copyright©2024, Wiley-VCH GmbH. (C) Thermo-responsive microparticles can be developed from DPTN crosslinked HA using emulsion technique which can be combined to develop thermos responsive granular hydrogels. These granular hydrogels can undergo programmed shape deformation post bio fabrication mimicking cartilage like constructs. (i) Schematic illustration of particle generation using emulsion approach, (ii) microscopic images showing thermo responsive nature of fabricated microparticles, reproduced from ref. 100 with permission. Copyright©2024, Wiley-VCH GmbH.

create granular hydrogels from hyaluronic acid-based hydrolytic microgels. When cultured with mesenchymal stem cell (MSC) spheroids, these microgels can give rise to dynamic cartilage tissue constructs (Fig. 2B).[99] More recently, temperature-responsive granular hydrogels have been developed using emulsification techniques, where norbornene-modified hyaluronic acid precursors were combined with a temperature-responsive crosslinker (dithiol-functionalized pNIPAM) to fabricate microparticles that exhibit dynamic shape changes post-3D printing, showing promise for cartilage tissue engineering (Fig. 2C).[100]

Although emulsification provides more precise control over particle size compared to extrusion fragmentation, it is not without its limitations. A significant drawback of the emulsification process is the involvement of oil and surfactants during particle formation. As a result, additional washing steps are often necessary to remove the oil phase, which can complicate the process. Furthermore, the technique depends heavily on surfactant concentrations and other fabrication parameters to achieve particles of the desired size. Although there is a clear correlation between surfactant concentration and particle size, excessive surfactant can lead to the formation of micelles, which are difficult to separate during washing stages. Also, emulsification is largely restricted to photopolymerizable hydrogels, making it less suitable for ionic crosslinked hydrogels. Ionic hydrogels are highly relevant in tissue engineering due to their unique stress-relaxation properties,[101,102] which more closely mimic the mechanical behaviour of biological tissues. Moreover, limited control on particle shape further limits the application of this particle generation approach to create granular hydrogels with unique micro-porous architectures.

Despite these challenges, emulsification remains a widely utilized and established method for the generation of microparticles, having been employed for several decades. The technique's simplicity, ability to efficiently control particle size, and compatibility with a wide range of both synthetic and natural hydrogel precursors make it an attractive option for the development of granular bioinks. Moreover, its high throughput capabilities and potential for translation from the laboratory to clinical applications are promising. The ability to fabricate smaller, more homogeneous particles makes emulsification a more favourable approach compared to fragmentation methods, and it holds significant potential for advancing the field of biofabrication, particularly for developing granular hydrogels for minimally invasive cartilage regeneration.

2.2.1.3 Micro-fluidics. Traditional batch emulsion methods for producing microparticles face several limitations, such as poor control over particle size, difficulty in fabricating non-spherical particles, and a heavy dependence on surfactant concentrations to regulate droplet size. These constraints spurred the development of controlled emulsion techniques using flow-focusing microfluidics in the early 2000s.[103] In these systems, oil and aqueous phases are guided to converge, where shear forces and hydrophobic effects create uniform aqueous droplets within the oil phase. By adjusting the geometry of the intersection and regulating the flow rates, highly monodispersed droplets can be produced. These droplets are then collected and processed for further applications.[104,105] Unlike traditional emulsion methods, which rely primarily on surface tension to form droplets, microfluidic-based droplet generation offers a more precise approach. The ability to modify microchannel dimensions and flow rates enables fine-tuning of droplet size and particle shape,[106] including the creation of non-spherical particles.[107,108] For instance, microfluidic technique can be employed to obtain microparticles with different shapes, including spherical and non-spherical microgels using a microfluidic flow-focusing device (MFFD).[109] The shape of microgels is determined by the volume of the droplet controlled by the flow rate of the continuous and disperse phases and the cross-sectional area of the outlet microchannel. The height and width of the microchannel should be larger than the diameter of the spherical droplets to obtain spherical microgels. Conversely, diameter of the spherical droplets being larger than any dimension of the outlet microchannel leads to the formation of non-spherical microgels, such as disk, ellipsoid, or rod-shaped.[109] Furthermore, incorporating multiple inlets into microfluidic devices allows for high-throughput generation of monodispersed particles (Fig. 3A).[110,111]

Microfluidics-based droplet generation has significantly advanced the manipulation of particle dynamics, allowing fine tuning of developed granular injectables. This approach provides a high level of control on the porosity of developed granular hydrogels by varying particle aspect ratio, shape, and size; a critical factor influencing the hydrogel's performance in regenerative medicine.[112] Recent studies have shown that particle shape can play a pivotal role in regulating angiogenesis, the process of new blood vessel formation.[82] Specifically, granular hydrogels developed using high aspect ratio micro-particles have been shown to promote organized vasculature growth, mimicking the structure of blood vessels *in vivo* and facilitating efficient angiogenesis (Fig. 3B).[113] This is a notable achievement, as controlling aspect ratios using batch emulsion techniques would be exceedingly difficult, if not impossible. Although cartilage is avascular and relies on diffusion from surrounding tissues for nutrient supply, damaged cartilage's regenerative capacity hinges on the ability of implanted materials to support controlled vascular ingrowth.[114] The precise control of particle size and shape enabled by microfluidics presents a promising strategy to optimize vascularization in cartilage repair, improving nutrient supply to chondrocytes and enhancing tissue regeneration.

Through microfluidics, researchers can precisely vary particle size and aspect ratio by adjusting the flow rates, enabling the development of granular hydrogels that are optimized for controlled angiogenesis.[82] Such controlled angiogenesis ensures speedy tissue regeneration, especially in tissues like cartilage, wherein extent of angiogenesis defines stem cell fate. Additionally, by manipulating particle size, researchers have been able to modulate the immune response to granular hydrogels, improving their biocompatibility.[115,116] This level of

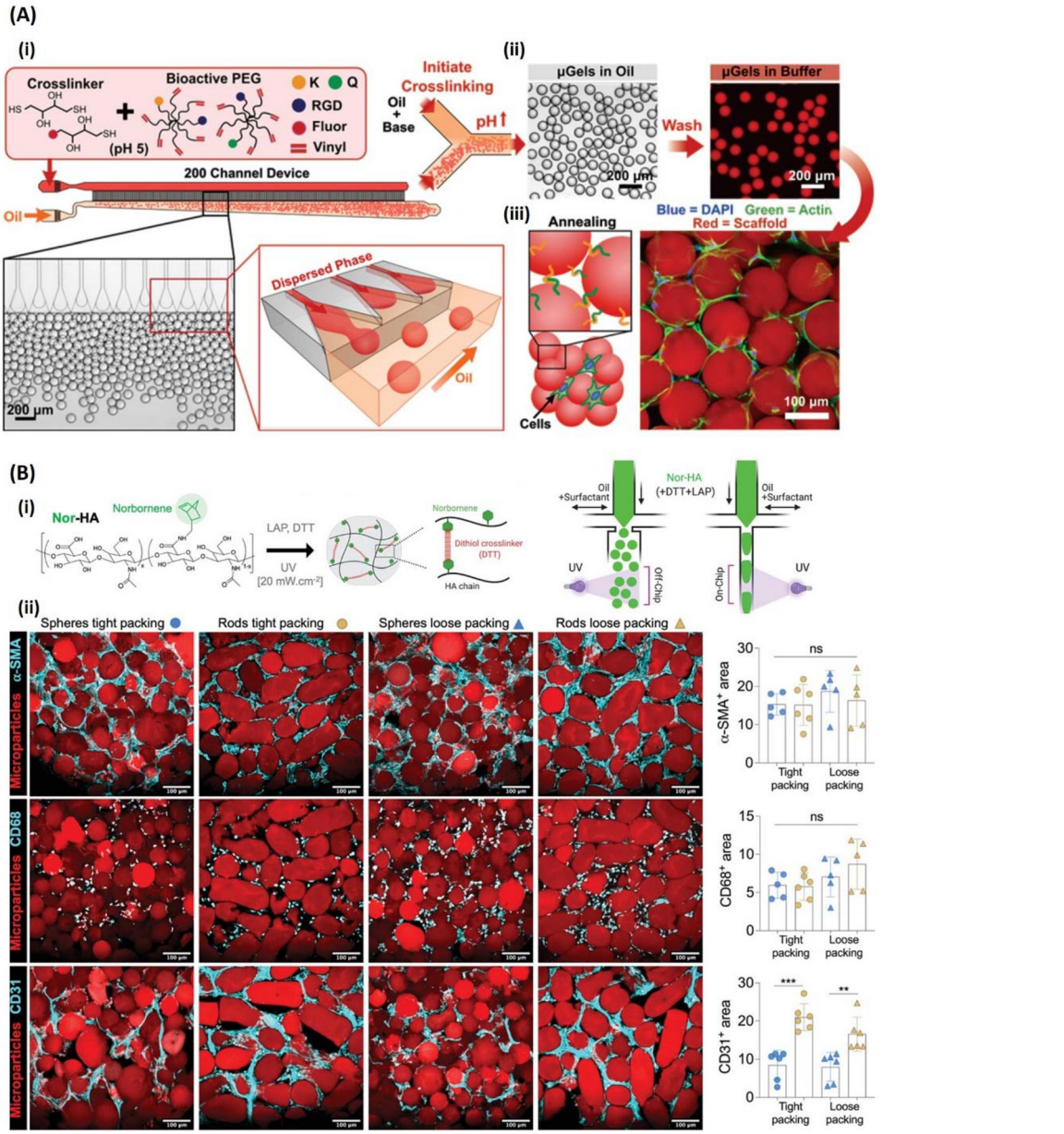


Fig. 3 Microfluidics-assisted microgel fabrication that can be utilized to develop granular hydrogel assemblies. (A) High-throughput particle generation using a parallelized step emulsification device. (i) Bioactive 8-arm PEG with dithiol, crosslinkers were utilized to fabricate microgels with on-chip gelation utilizing pH *via* Michael addition reaction, (ii) fabricated microparticles post washing and suspending in PBS, (iii) annealed microparticles into micro-porous assemblies allowing cell infiltration through micropores, reproduced from ref. 110 with permission. Copyright©2019, Wiley-VCH GmbH. (B) Non-spherical particle generation using droplet-based microfluidics that can be utilized to control the performance of granular hydrogels. (i) Nor-HA microgels with different aspect ratios fabricated using microfluidics, (ii) performance of granular hydrogels based on particle shape upon subcutaneous implantation, demonstrating efficiency of non-spherical particles in controlling host cell infiltration and modulating immune response, reproduced from ref. 113 with permission. Copyright©2022, Wiley-VCH GmbH.

control has led to improvements in the injectability and overall performance of these hydrogels, ensuring homogeneity in the formulation of granular hydrogels and making them more suitable for clinical applications.

Despite these advancements, microfluidics-based droplet generation does have certain limitations that hinder its widespread application. One of the main drawbacks is the relatively low throughput of most microfluidic droplet generation systems, which limits their scalability for the large-scale production of granular hydrogels. Furthermore, although particle aspect ratios can be controlled to some extent, the precision in shaping microparticles remains a challenge. Most systems still predominantly produce spherical particles, which restricts the exploration of the full potential of microfluidics for producing

diverse particle shapes. Given the significant impact of particle geometry on the performance of granular hydrogels, this limitation prevents the technique from being fully exploited.

Although some challenges exist, microfluidics remains one of the most precise methods for generating homogeneous microparticles. Its capability to produce heterogenous granular hydrogels has made it the most widely explored approach for such applications. Microfluidics is particularly advantageous due to its ability to control particle size and generate droplets from ionically crosslinked hydrogels. Additionally, the high biocompatibility of microfluidics-assisted droplet generation facilitates cell encapsulation during the droplet formation process. This allows for the creation of cell-encapsulated granular hydrogels, which can be further mixed with secondary cell types, facilitating the development of co-culture systems for advanced tissue engineering applications. Furthermore, with the advent of state-of-the-art additive manufacturing techniques, researchers can now design custom microfluidic devices with unique channel geometries, enabling the generation of microparticles with tailored shapes and sizes, thereby opening new possibilities for fabricating functional biomaterials with precisely controlled properties.

2.2.1.4 Other approaches. Microfluidics-based methods have demonstrated the ability to generate microparticles with varying aspect ratios; however, their limitations in precisely controlling particle shape and size, coupled with low throughput production rates, have prompted researchers to explore alternative bottom-up fabrication strategies. In recent years, techniques such as complex coacervation, in-air microfluidics, and electrohydrodynamic (EHD) spraying have been widely employed to produce microgels. For example, in-air microfluidics enables the formation of granular alginate-based hydrogels and offers higher particle production rates, which have shown great promise in applications such as 3D bioprinting and minimally invasive injectable therapies (Fig. 4A).[117,118] It involves the collision of two streams, one with the hydrogel precursor and the other with the crosslinker in air, which leads to the formation of droplets in air. EHD spraying operates by the application of voltage to the hydrogel precursor while it extrudes through a nozzle, leading to a jet of charged droplets collected over a grounded bath and later crosslinked to form microgels. It can generate monodisperse microgels with fine control over particle uniformity and composition and is compatible with cell encapsulation. For example, alginate microgels can be fabricated by jetting into a calcium chloride bath (Fig. 4B).[119] Additionally, complex coacervation, which is based on the principle of phase separation of two oppositely charged polymers in an aqueous solution, has been employed to fabricate composite microparticles. For instance, GelMA was combined with modified alginate, wherein the charge differential-induced phase separation leads to formation of microdroplets that can be crosslinked to form microgels (Fig. 4C).[120]

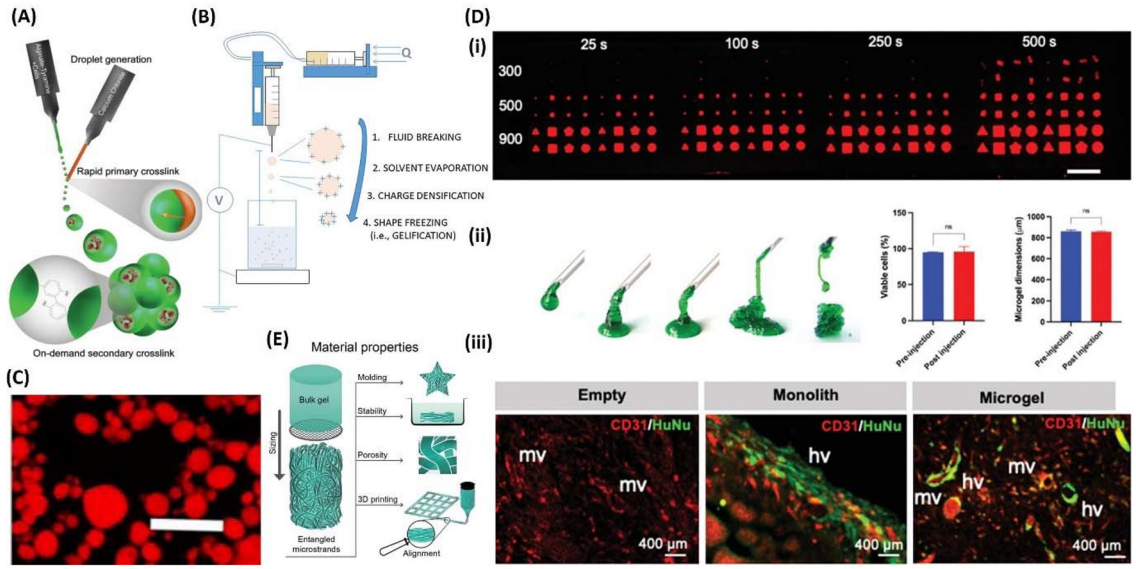


Fig. 4 Generation of microgels using other approaches. (A) In-air microfluidics jetting approach to generate uniform alginate beads that can be utilized for developing granular hydrogels, reproduced from ref. 118 with permission. Copyright©2014, Wiley-VCH GmbH. (B) Electrohydrodynamic spraying approach to create uniform microparticles from hydrogel precursors, reproduced from ref. 119 with permission. Copyright©2021, Wiley-VCH GmbH. (C) Complex coacervation approach to create uniform GelMA droplets, reproduced from ref. 120 with permission. Copyright©2015, Wiley-VCH GmbH. (D) Layer-by-layer fabrication of non-spherical microgels using a DLP 3D printer. (i) Possible dimensions of microgels that can be fabricated, (ii) injectability of microgel assembly showing easy extrusion through a 21G nozzle, (iii) microgel assembly allowing efficient vascular infiltration compared to bulk counterparts (monolith), whereas no host cell infiltration was observed in empty cavities, demonstrating the potential of microgel assemblies in accelerating the new vascularization and tissue infiltration, reproduced from ref. 125 with permission. Copyright©2023, Wiley-VCH GmbH. (E) Micro-strands shaped high aspect ratio microparticle generation using simple pressing of bulk hydrogels through microgrids, reproduced from ref. 130 with permission. Copyright©2020, Wiley-VCH GmbH.

These bottom-up fabrication methods provide enhanced material versatility and have expanded the design space for microgel-based systems. However, while they enable some degree of customization in microparticle synthesis, they often face challenges in achieving precise and consistent control over both particle morphology and size at scale. This has fueled ongoing efforts to refine these techniques and combine them with advanced engineering strategies for improved microparticle production. Stereolithography has emerged as a versatile technique to generate non-spherical microgels using templated molds and photomasks to control microgel geometries.[121] In case of imprint lithography, a hydrogel precursor is loaded into a templated mold with the negative features of the desired microgels with the subsequent crosslinking and curing within the mold to obtain microgels with desired features. Alternately, a templated photomask can be employed to cover a hydrogel precursor solution while photo crosslinking, thereby selectively crosslinking the area not covered by photomask into microgels. The fabricated microgels are then collected after removing the uncross linked hydrogel precursor. Flow lithography offers advantages of increased yield through continuous production of microgels, wherein a hydrogel precursor solution flows through a channel and photomasks are used to cure regions of the precursor solution at regular intervals to form microgels. The desired geometry of the microgels can be obtained by altering the photomask used to cure the hydrogel precursor solution.

Recently, emerging maskless lithography approaches has further advanced the control and the challenges associated with mask based approaches.[122,123] The development of cutting-edge maskless lithography techniques, particularly Digital Light Processing (DLP), has significantly enhanced the precision in using photo-polymerizable hydrogels to fabricate microparticles with well-defined shapes and sizes. These advanced DLP-based lithography methods now allow to produce hydrogel structures at the microscale, expanding the capabilities of current fabrication technologies. This allows for greater control over the geometry and structure of the particles, offering new possibilities in the field of microfabrication (Fig. 4D).[47,124,125] One of the significant advantages of maskless approaches is their ability to utilize computer aided designs (CAD), which can allow high control on particle shape and dimensions.[126] By manipulating particle shapes and geometries, this technology can enable the creation of injectable, micro-porous hydrogels with unique properties tuned for a particular tissue type. Non-spherical particles generated using lithography approaches can act as micro-niches to control various cellular processes; such as differentiation, alignment, ECM secretion, and other therapeutic cell response;[121] while also allowing fine tuning of granular hydrogel porosities to great extent.[123] Such control on cell behaviour by controlling micro-particle geometries can improve the performance of granular hydrogels when used in medical applications.

Apart from lithography, wet spinning is another widely explored technique to generate high aspect ratio microfiber-gels by extruding the hydrogel precursor solution in a syringe into a solution bath containing crosslinkers to the hydrogel precursor. The crosslinking of hydrogel precursor solution proceeds leading to the formation of hydrogel microfibers. For example, microribbon-like microgels of gelatin were fabricated by ejecting gelatin/dimethyl sulfoxide solution using a pump at room temperature into a bath of anhydrous ethanol (EtOH).[127] However, multiple post-processing steps are involved, such as further drying in acetone, microribbon dissociation and washing with ethanol, methacrylation with methacrylic anhydride, fixation with 0.1% glutaraldehyde, washing with deionized (DI) water, and neutralization with L-lysine hydrochloride in phosphate-buffered saline (PBS) to finally achieve microribbon-gels. However, suitable modifications in the technique can avoid the need for post-processing steps. Wet spinning was employed to eject a composite solution containing SA, MeHA macromolecules, and the photoinitiator lithium phenyl-2,4,6-Trimethylbenzoylphosphinate (LAP) into a calcium chloride ($CaCl_2$) coagulation bath.[128] This led to the immediate crosslinking of the SA macromolecules within the composite precursor fibers, thereby forming partially crosslinked microfiber gels. These microfiber gels were collected, dried, free-stacked in a mold, and can be further crosslinked in the presence of the photoinitiator LAP and UV light, thereby avoiding the need for any post-treatment.

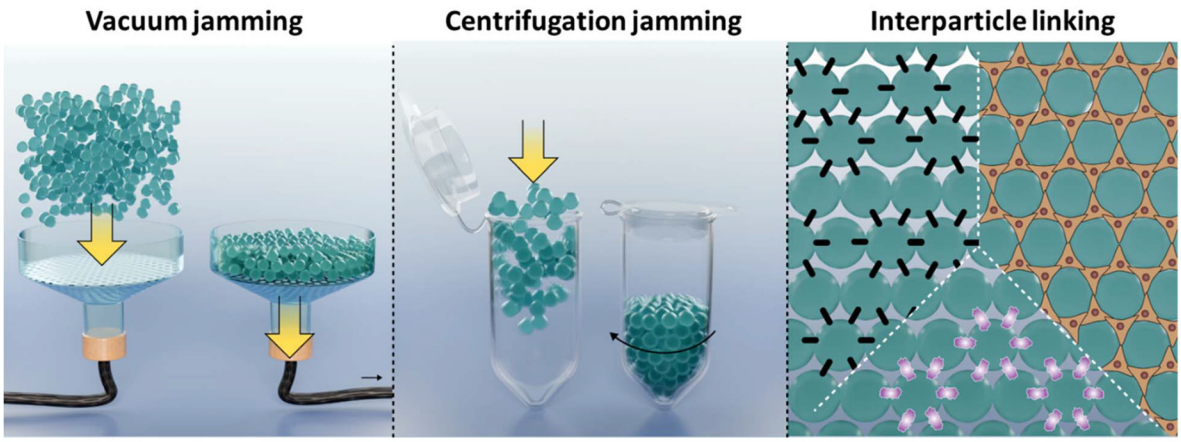
Microfluidic spinning method, based on the standard microfluidic technique, is yet another technique employed for producing hydrogel microfibers with or without cells.[129] This method utilizes multiple injection capillaries coaxially aligned within a collection capillary. The injection capillaries are infused with the hydrogel precursor solution while the crosslinking solution is pumped in the same direction into the collection capillary. This design leads to *in situ* generation of hydrogel microfibers due to the hydrodynamic focusing effect, wherein a 3D coaxial sheath flow stream forms around the flow of the precursor solution, and crosslinking occurs when the two flows meet at the merging point. The morphology of the microfiber gels can be precisely controlled by the configuration of the injection capillaries. Apart from microfibers, microstrands-based granular hydrogels are also reported as another class of injectable materials, with microstrands-shaped microparticles helping in creating granular injectables mimicking anisotropic microenvironments suitable for cartilage tissue engineering. Entangled microstrands can be created by pressing a bulk hydrogel through a grid with micron-sized apertures to deconstruct the hydrogel into individual microstrands (Fig. 4E).[130] The production is fast, needing no specialized instruments, and can be utilized with litre volumes of hydrogels, a high-throughput fabrication approach for generating high aspect ratio microparticles.

Hydrogel microparticles and microfibers/microstrands offer significant potential for engineering granular hydrogels tailored for cartilage tissue regeneration. Despite these advancements, several limitations persist. In-air microfluidics and coacervation methods still face challenges in consistently generating non-spherical or highly complex geometries. Lithographic techniques, while precise, often require expensive equipment,

slower production speeds, and in some cases, photo initiator toxicity concerns. Wet spinning methods typically involve labour-intensive post-processing steps that reduce scalability, though recent improvements have mitigated some of these limitations. Also, though microstrand generation offers potential for high-throughput production of high aspect ratio microparticles, it remains a relatively underexplored method for particle generation, with majority of researches still relying on microfluidics, fragmentation, and emulsification as more preferred methods. Moreover, ensuring biocompatibility, mechanical strength, and integration with host tissue remains a critical challenge, especially when translating these microgels into clinical settings.

2.2.2 Jamming approaches: From hydrogel microparticles to injectable microporous assemblies. Jamming refers to the process of compacting hydrogel micro-particles into tightly packed assemblies with interstitial spaces between them.[131,132] This process induces a transition of the micro-particles from a fluid-like state to a more rigid, solid gel-like state, while enhancing the material's extrudability. The jammed assemblies exhibit shear-thinning behaviour, making them ideal for use as micro-porous granular injectables.[133] The outcome of this jamming process is highly dependent on the shape and size of the particles, as well as the specific method employed to compact them.[98,134] These factors play a critical role in determining the mechanical properties of the resulting granular hydrogel and its performance in applications such as regenerative medicine. Various strategies have been employed to achieve jamming of hydrogel microparticles;[135] however, in the context of this review, which centers on cartilage tissue engineering, we highlight the most prominent methods. These include: (i) vacuum-induced jamming, (ii) jamming through the application of centrifugal forces, and (iii) interparticle linkage through supramolecular chemistry or covalent linking of microparticles (Scheme 3 and Table 2). The following sections will provide a brief overview of each of these jamming approaches.

2.2.2.1 Vacuum jamming. Among one of the simplest approaches for annealing micro-particles, vacuum jamming has been widely utilized to successfully develop granular counterparts of bulk hydrogels.[62] The technique relies on exposing fabricated micro-particles to a vacuum filtration setup, effectively removing excess fluid and allowing particle packing while maintaining interstitial spaces that allows efficient diffusion of nutrients and cellular migrations when these hydrogels are used as injectables.[92] During the process, capillary forces drive physical interactions and mild adhesions at the interfaces, leading to a jammed yet extrudable structures



Scheme 3 Schematic representation of various jamming approaches with a summary of each approach tabulated below (Table 2).

Table 2 Summary of major jamming approaches utilized for microgel packing into granular bulk hydrogel

Approach	Mechanism	Porosity control (void fraction)	Stability of granular injectable	Utilization for MIPs	As bioinks
Vacuum jamming	Removal of interstitial air in a confined chamber for microgel compaction	~20–40% (tunable by vacuum intensity)	Moderate (dependent on vacuum strength)	Moderate-gentle compaction, limited shape fidelity	Limited with secondary crosslinking often required for stabilizing the structures
Centrifugation jamming	Force-driven compaction	~10–30% (adjustable by speed/time)	Moderate-high (higher speed yields stronger packing)	High-controllable packing	Moderate-high-useful with supportive polymers
Interparticle linking	Covalent or supramolecular linking	~15–35% (varies with linker density)	High (chemical bonded networks, stable gels)	High-retains structure post injection	High-suitable for direct bioprinting

with shear thinning properties.[85] By controlling the particle shape, polymer concentrations, and vacuum pressure, tuneable void spaces can be generated in developed granular hydrogels.[136]

The simplicity and versatility of vacuum jamming have made it a popular technique for creating shear-thinning injectable granular hydrogels from a broad range natural and synthetic polymers. For instance, methacrylate oxidized alginate (OMA) particles were utilized to develop granular hydrogel bioinks that can be further stabilized with light post extrusion due to presence of methacrylate groups. Developed granular hydrogels were utilized as bioink for 4D bioprinting and demonstrated tremendous potential for chondrogenesis due to polysaccharide matrix as its backbone.[90] Similarly, researchers have utilized vacuum jamming process to develop injectable granular hydrogels from Gelatin methacryloyl (GelMA) and Oxidized Alginate (AlgOx). Microparticles of GelMA and AlgOx were synthesised utilizing extrusion fragmentation and centrifugal microgel device respectively, and were jammed using vacuum filtration. By tuning the void fractions in jammed hydrogels, the authors showed their capacity to tune cellular response.[137] A comparable approach has been employed to create granular hydrogels from Norbornene modified Hyaluronic acid (Nor-HA) microparticles fabricated using microfluidics (Fig. 5A). By tuning the polymer compositions and particle size, researchers demonstrated the impact of variable void spaces (micro-pores) on cell behaviour with possible extension of this approach to a wide range of natural and synthetic polymeric micro-particles.[80] Recently to enhance the potential of granular bioinks, platelet lysates have been coupled with hydrogel microparticles to enhance their regenerative potential. For instance, Nor-HA was mixed with Platelet lysate and clotting factors to create microparticles with fibrillar network using microfluidics.[138] These microparticles were then jammed using vacuum jamming to create granular hydrogels that showed native ECM like fibrillar architectures. Developed hydrogels showed high extrudability and shear thinning properties and showed potential as micro-porous fibrillar hydrogels for minimal invasive treatments.

Latest advancements in the area have driven the exploration towards creating granular hydrogels with multi-functionality. For instance, in regenerative medicine, electroactive properties have known to enhance the performance of scaffolds/injectables upon implantation.[139,140] Electrical stimulation can enhance the regenerative potential of bulk hydrogels and have been largely explored for different areas of regenerative medicine.[141,142] Recently, electroactive granular hydrogels have been explored as a promising alternative over bulk counterparts. By fragmenting bulk hydrogels into microparticles, enhancement in conductivity and improved extrudability are possible advantages along with micro-porous architectures. This has been validated in recent research, where authors

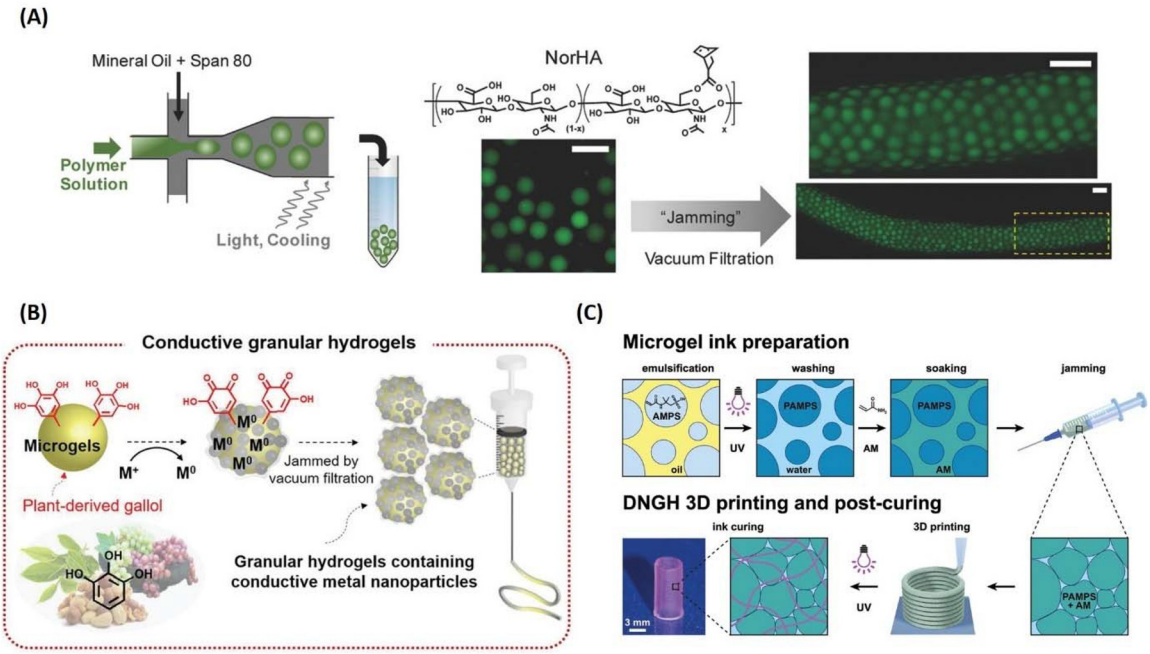


Fig. 5 Vacuum driven jamming allowing development of microporous granular hydrogels from micro-particles. (A) NorHA microparticles fabricated using microfluidics can be jammed into granular hydrogels with injectability and shear-thinning properties, reproduced from ref. 62 with permission. Copyright©2019, Wiley-VCH GmbH. (B) Electroactive granular injectables can be developed by jamming conducting microgels using vacuum filtration. Gallol modified hyaluronic acid conducting microparticles showing injectability and shear thinning properties post vacuum driven jamming, reproduced from ref. 143 with permission. Copyright©2019, Wiley-VCH GmbH. (C) Polyelectrolyte-based double network granular hydrogels with fascinating mechanical properties mimicking soft tissues can be developed using vacuum-driven jamming, reproduced from ref. 144 with permission. Copyright©2020, Wiley-VCH GmbH.

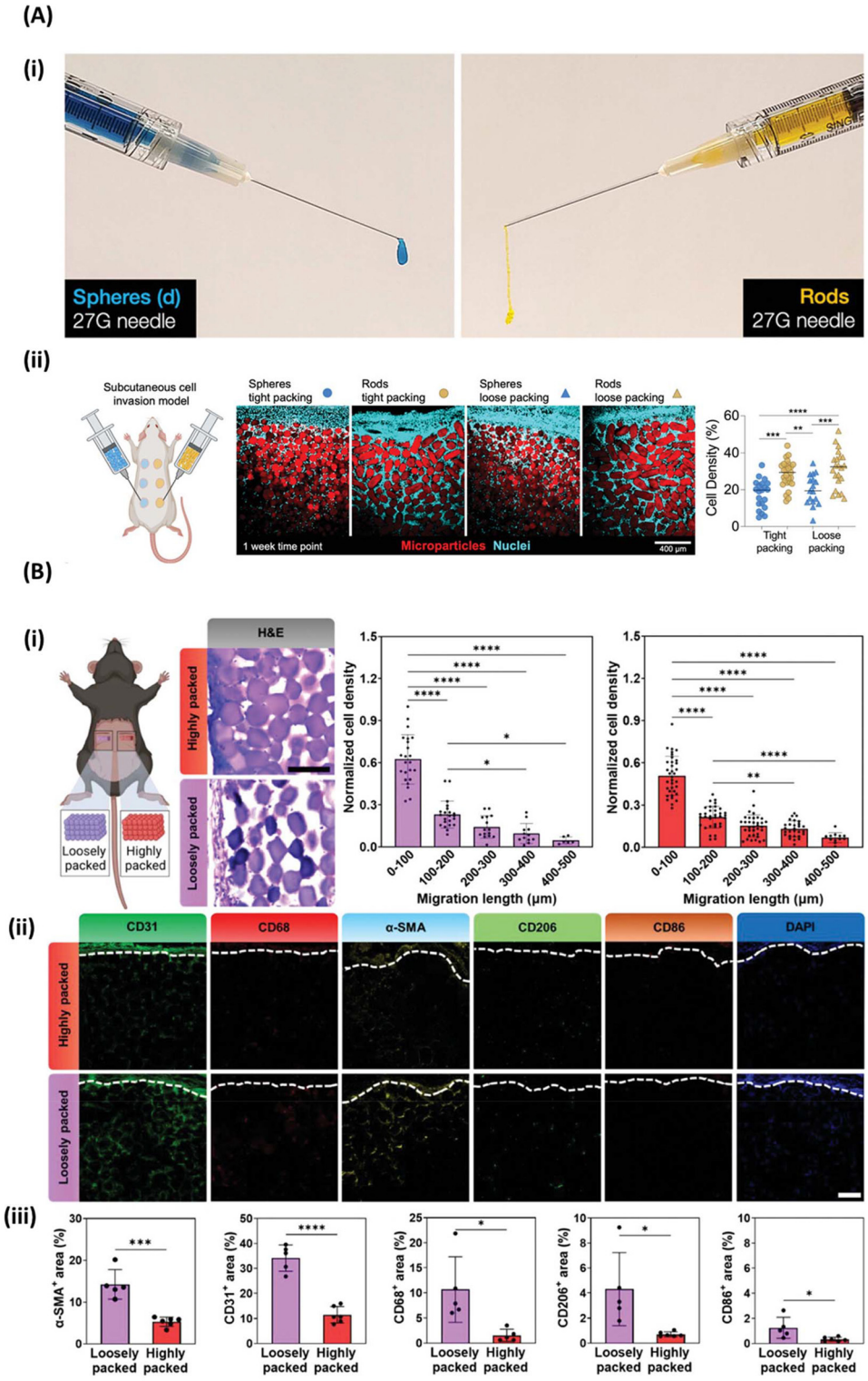


Fig. 6 Centrifugation driven development of granular microporous assemblies with adaptive porosities by variation of centrifugation speed. (A) Norbornene modified hyaluronic acid microrods and microspheres packed using a centrifugation-driven approach into bulk granular hydrogels. (i) Injectability evaluation of granular hydrogels made up of rods or spheres through a 27G needle, (ii) impact of variation of centrifugation speed on packing density and corresponding host cell infiltration upon subcutaneous implantation, reproduced from ref. 113 with permission. Copyright©2022, Wiley-VCH GmbH. (B) *In vivo* response of varying centrifugation speed on granular hydrogel properties upon subcutaneous implantation. (i) Histological examination of loosely and highly packed granular hydrogels upon subcutaneous implantation, demonstrating that control on cellular infiltration can be achieved through precisely controlling the microporosities by variation in centrifugation speed. (ii) Immune cells and neo-vascularization infiltration depths in granular hydrogels of varying porosities. (iii) Quantified values depicting precise control on injectable properties by varying microporosities, reproduced from ref. 116 with permission. Copyright©2024, Wiley-VCH GmbH.

developed a shear thinning granular hydrogels from gallol modified Hyaluronic acid (HA-Ga) microparticles fabricated using microfluidics. Post fabrication, particles were modified *via* an *in situ* metal reduction process and were subsequently jammed to create injectable granular hydrogels with electroactivity that can be utilized for tissue regeneration (Fig. 5B).[143] It is well known that electric potential accelerates the proliferation of chondrocytes and the synthesis of ECM molecules that can accelerate the regeneration process. In this regard such electroactive granular hydrogels have tremendous potential for their exploration in treatment of cartilage disorders. Recently, the concept of double network granular hydrogels can further fine tune the mechanical properties of these granular composites, extending their potential in soft tissue engineering. These granular composites can be easily developed using vacuum jamming process and have shown potential to support cellular growth (Fig. 5C).[144]

Vacuum jamming processes allows developing granular bioinks from a wide variety of synthetic and natural polymers, since no chemical modifications are required for jamming them. This has allowed researchers to explore granular hydrogels with multi-functionality such as electroactivity, fine tuning of mechanical properties, and possible inclusion of other tissue specific polymers to extend their utilization in wide range of soft tissues. Despite the rapid adoption of vacuum jamming in the creation of granular assemblies, the technique has inherent limitations, primarily due to its reliance on vacuum forces. The stability of the developed bioinks is highly dependent on the vacuum pressure applied during the jamming process. Low vacuum levels can result in unstable granular assemblies, increasing the likelihood of hydrogel particle disintegration when injected for regenerative purposes. Conversely, excessive vacuum pressure can deform the hydrogel particles, reducing the available pore space and limiting the diffusion potential within the granular bioinks. Furthermore, controlling porosities; an important aspect particularly when applications are avascular tissues like cartilage, vacuum jamming approaches provide limited control on achieving controlled porosities. Although these trade-offs restrict the broader application of vacuum jamming, the technique remains widely used due to its simplicity, versatility, and ability to create granular hydrogels from a diverse range of natural and synthetic microparticles with minimal modification.

2.2.2.2 Centrifugation driven – jamming. Centrifugation-driven jamming of micro-particles is a widely adopted technique for creating granular hydrogels, as it allows for the easy formation of densely packed hydrogel assemblies.[145] By applying centrifugal forces to micro-particles, the repulsive interactions between them are reduced, enabling the particles to aggregate and form structures with interstitial spaces. These aggregated particles can then be easily collected as granular aggregates and utilized in various applications, ranging from regenerative medicine to granular bioinks for 3D/4D printing. One of the key advantages of centrifugation jamming is its versatility, particularly in controlling the void spaces between particles by adjusting the centrifugation speed (Fig. 6A).[113,116] This enables researchers to generate granular assemblies with a wide range of void spaces, providing insight into how these voids influence the mechanical properties and cellular responses of granular injectables.[21]

Such controls on porosities particularly makes this technique extremely useful for tuning granular hydrogels for tissues like cartilage.[146,147] Cartilage is an avascular tissue, which makes diffusion a critical factor in its regeneration. In this context, optimizing the void space within granular hydrogels ensures adequate nutrient and oxygen diffusion, supporting the migration of cells and promoting efficient tissue repair. Centrifugation jamming has enabled the creation of anisotropic granular assemblies, with controlled vascular patterns and cellular infiltration, by fine tuning void spaces.[134,148] Such assemblies can be extended to injury models, providing valuable insights into how the void spaces within granular hydrogels impact tissue recovery. A similar approach has been applied to study the effect of packing density in GelMA-based granular hydrogels, where researchers have demonstrated how varying centrifugation speed affects cellular behaviour and *in vivo* hydrogel performance.[116] Adjusting packing density can fine-tune macrophage polarization (Fig. 6B),[116,149,150] further highlighting the potential of this technique in optimizing hydrogel properties.[151] Recent advancements have incorporated nano-silicates with hydrogel microparticles, to further fine tune interconnectivity of void spaces.[152] Researchers have shown that the addition of negatively charged nano-silicates can further refine the void structure, enhancing cell penetration and supporting the deeper migration of cells into the hydrogel.[152] This combination allows better fine-tuning of micro-porosities and enhances the regenerative potential of these hydrogels.

Although centrifugation-based jamming has proven valuable in optimizing the micro-porosity of granular hydrogels, it also introduces challenges. The process can lead to heterogeneity in the packing of particles, resulting in regions with either loosely packed or densely packed particles. Furthermore, the high centrifugal forces applied during the process may deform delicate microparticles, potentially compromising the hydrogel's structural integrity. The centrifugation procedure can also be time-consuming, and the heat generated during the process could affect temperature-sensitive microparticles. Despite these drawbacks, the simplicity and precision with which centrifugation can control void spaces make it a widely used technique for investigating and developing granular hydrogels with tailored properties, particularly in the context of regenerative medicine.

2.2.2.3 Interparticle linkage. Jammed hydrogels developed using approaches such as vacuum and centrifugation driven jamming interact through non-specific physical forces, which can easily deform upon mechanical stress, limiting their application for load-bearing tissues such as cartilage. Moreover, monodisperse granular hydrogels developed using such approaches often fails to recapitulate the *in vivo* complexities. In contrast, the granular hydrogel assembly *via* interparticle linkages can develop heterogenous granular assemblies[153] and

3D environments that can closely recapitulate such complexities. Introducing supramolecular or chemical modifications in these hydrogels can endow dynamic properties such as mechanical reinforcement, elasticity, self-healing, etc.[48,154] Granular hydrogels can be tuned across a vast range by manipulating a wide spectrum of supramolecular and chemical linkages. The supramolecular interactions include hydrogen bonding, electrostatic and host–guest interactions, *etc*. Hydrogen bonding is a relatively weak physical interaction, but increasing the hydrogen bonding density can give mechanically sound structures. Host–guest linkage is a non-chemical interaction in which a function moiety like adamantane is physically entrapped in the host cavity (like cyclodextrin). Hydrogels formed by these interactions have good self-healing and shear-thinning properties. For example, Joshua E. Mealy *et al.* fabricated a granular hydrogel assembly using the host–guest interactions between the hyaluronic acid microgel (Fig. 7A).[70] Interparticle linkages induced by host–guest interactions provided mechanical resiliency to the hyaluronic acid granular hydrogels. Host–guest interactions are usually responsive to certain stimuli, which can be varied with the responsive nature of the guest to a specific stimulus. These interactions are generally fast and biocompatible.[155] Another approach is to use granular hydrogels carrying either positive or negative charge that can assemble due to the electrostatic interactions at the interface.[156] The electrostatic interactions are dynamic and reversible in nature. Environmental conditions like acidic or alkalinity can affect the assembly and disassembly of granular hydrogels. Zaman Ataie *et al.* used a unique approach to introduce jamming the granular hydrogels by using charge-driven silica nanoparticles, which can form dynamic bonds between the microgel particles.[152] In another study, polymers with opposite charges were used to fabricate microgel assembly. Gelatin methacrylate and chitosan methacrylate carry negative and positive charges, respectively, mediating the formation of microgel assembly through electrostatic interactions (Fig. 7B).[157]

Besides leveraging supramolecular interactions for granular hydrogel assembly, reversible chemical interactions can also be utilized for interparticle linkages. Chemical reactions between the functional moieties present in the microgels drive such interactions and offer stability to the granular hydrogels.[158] The ways through which chemical interactions can be introduced are click chemistry,[159] Schiff bases,[84] enzymatic linkages,[160] and photo-initiated radical polymerization.[116] Click chemistry mediates microgel assembly through heteroatom linkages. The click chemistry can be thiol–ene, Diels–Alder, or vinyl sulfone-amine reactions, which are highly selective and give high yields (Fig. 7C). Granular hydrogels can also be assembled using enzymes as a catalysis. For instance, two peptide-grafted polyethylene glycol-based microgels were linked *via* the formation of amide bonds between the peptide ends facilitated by the enzyme transglutaminase factor XIIIa.[161] The photo-initiated polymerization reaction involves the generation of free radicals under the presence of light, which helps in the polymerization of the reactive group to induce a bond and, ultimately, microgel assembly. Christopher B. Highley *et al.* employed norbornene-modified hyaluronic acid and acrylate polyethylene glycol to fabricate jammed microgels using a photo-mediated polymerization reaction.[62] Another study synthesized microgel using ionic crosslinking of alginate methacrylate and microgel assembly using Photo crosslinking.[162]

The interparticle-driven microgel jamming method is a well-established approach for creating granular hydrogel assemblies, but it is not without its challenges. One significant limitation is that it often requires the modification of pre-polymers with specific functional moieties. This adds an extra layer of complexity to the process, making it more time-consuming and potentially hindering scalability. The incorporation of these functional groups typically demands specialized chemical steps that are difficult to standardize for large-scale production, raising concerns about the commercial viability of this approach. Additionally, in many cases, secondary stabilization methods such as vacuum treatment or centrifugation are employed to maintain the structural integrity of the granular assemblies, introducing additional complexities to the method, which can further limit its practicality.

Another critical challenge associated with this method is the difficulty in precisely controlling the pore size within the resulting hydrogel structure. The size and distribution of pores are essential for tailoring the material's properties, such as its mechanical strength, drug delivery capabilities, or its suitability for specific biological applications. However, the interparticle jamming mechanism, which depends on the interactions between functionalized microgels, often leads to unpredictable pore formation, limiting the control over these structural characteristics. This lack of precise pore size control can hinder the performance of the hydrogel in certain applications, particularly in sensitive areas like tissue engineering, where uniformity and reproducibility are crucial.

Despite these drawbacks, the chemical modifications used in the interparticle linking approach do confer certain advantages, particularly in terms of the mechanical strength and self-healing properties of the resulting granular assemblies. These enhancements make the approach particularly promising for applications where robust, load-bearing materials are required, such as in the development of artificial cartilage or other tissue-engineering applications. The self-healing ability is a desirable feature for applications that require long-term functionality under mechanical stress. However, the need for chemical modifications and the associated complexities makes this approach less attractive when scalability and ease of control are prioritized.

3 Application in cartilage repair and regeneration

3.1 Granular hydrogels in cartilage tissue engineering

The purpose of cartilage tissue engineering (CTE) is to repair the damaged region with cell and tissue regeneration to

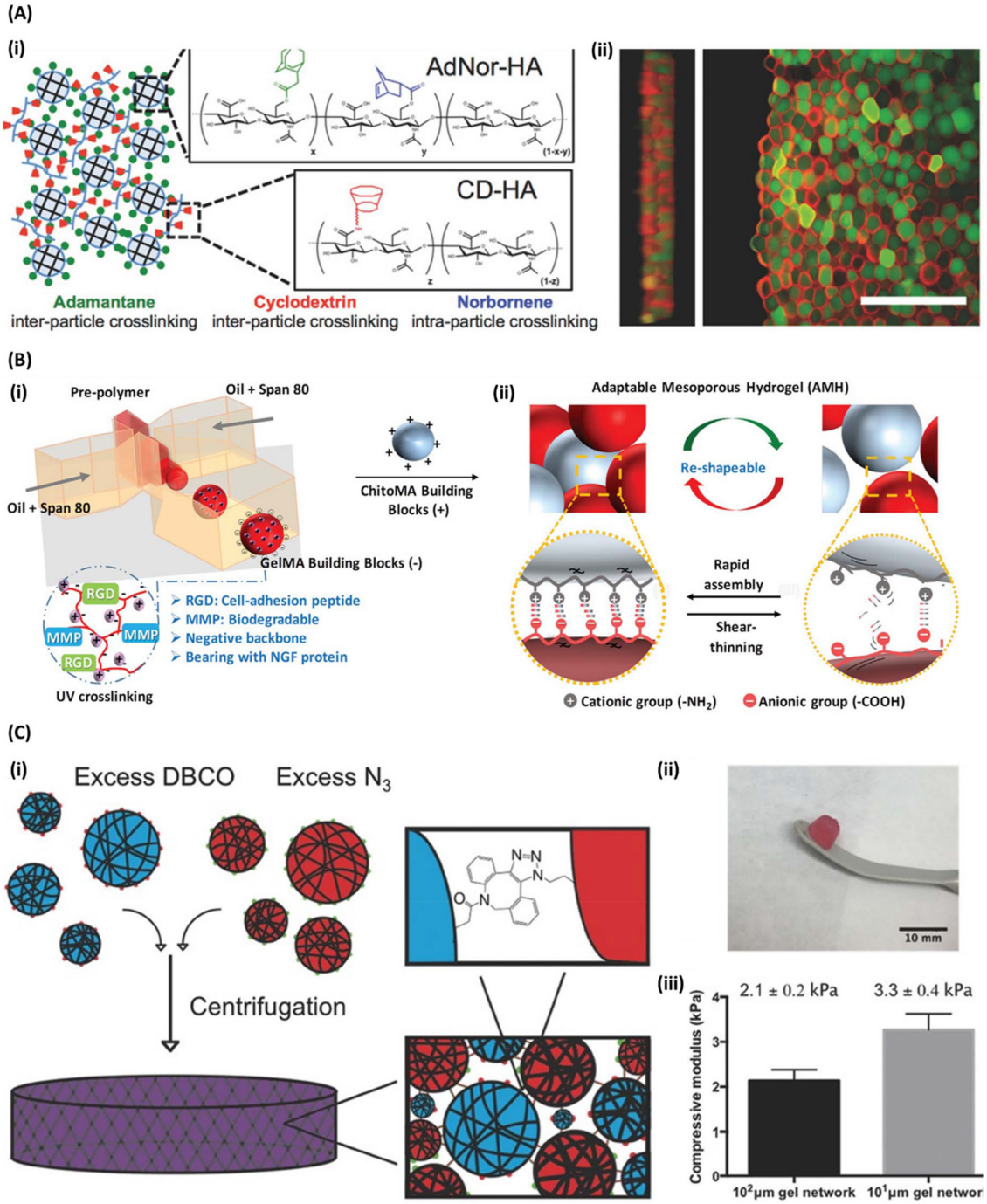


Fig. 7 Microgels linking through chemical modifications. (A) Host–guest interaction-driven jamming of microgel particles. (i) Adamantane modified Norbornene HA (guest) and cyclodextrin modified Norbornene HA (host) microgels were fabricated using droplet-based microfluidics. (ii) Host–guest interactions allowed jamming of microgels into porous injectable assemblies, reproduced from ref. 70 with permission. Copyright©2018, Wiley-VCH GmbH. (B) Electrostatic-driven jamming of microgels. (i) Negatively charged methacrylated chitosan and positively charged methacrylated gelatin droplet generation using microfluidics. (ii) Electrostatically jammed microgels can then be utilized as granular injectables, reproduced from ref. 157 with permission. Copyright©2019, Wiley-VCH GmbH. (C) Click chemistry-driven assembling of PEG microgels. (i) DBCO and N_3 modified PEG microparticles generation using microfluidics. (ii) Click chemistry-driven microgel jamming resulting in the formation of a granular microporous assembly. (iii) Mechanical properties of granular assembly developed using PEG microparticles of different diameters, reproduced from ref. 159 with permission. Copyright©2017, Wiley-VCH GmbH.

restore the structural and biological functions of cartilage. Granular hydrogels are promising in CTE because of their distinguishing features, such as well-defined micro-scale porosities, interconnected pores, tuneable biodegradability, and mechanical properties.[130] In particular, the self-healing and shear-thinning properties of these hydrogels sanction the ease of injectability at the injury site in a minimally invasive way.[48,163] Further, using granular hydrogels allows the production of spatial heterogeneity in the 3D printed construct to mimic the hierarchical and spatial organization of cells and ECM components as present in the native cartilage. This is achievable by mixing different populations of hydrogel microparticles with distinct physical and mechanical features, like stiffness, to create heterogeneity. The unique advantages of granular hydrogels are that they provide better printability due to excellent shear thinning and self-healing mechanisms, and optimal cellular functions due to microporous structure, providing multifunctional properties to the bioinks for 3D bioprinting of cartilage mimicking scaffolds.[92,135,164] Herein, we have discussed the application of granular hydrogels for cartilage tissue engineering.

3.1.1 Granular hydrogel as injectable biomaterials for cartilage tissue engineering. MIPs for treating partial thickness chondral defects offer distinct advantages, such as direct delivery of hydrogels and compounds at the site, reduced risks of complications, faster recovery, minimal scarring at the site of injection, and the ability to self-fit in the irregular defect.[29] Granular hydrogels can prove to be an excellent candidate for MIPs due to their superior rheological properties and injectability. The self-healing behaviour of granular hydrogels further strengthens this theory as it will restore the structural composition of the hydrogel post-injection. Like the traditional hydrogels, granular hydrogels can be reinforced with cells like stem cells or autologous chondrocytes or bioactive compounds, including drugs and growth factors, to accelerate healing of cartilage lesions *via* enhanced chondrogenic induction and/or immunomodulation.[70] In this section, we have reviewed and discussed the efforts made in the design and development of granular hydrogels with multifunctional properties as injectable materials for MIPs to treat cartilage degenerative diseases. Conrad *et al.* designed gelatin-based microribbon (μRB) granular hydrogels with increased mechanical properties to enhance cartilage regeneration (Fig. 8A).[165] The porous structure imparted shock-absorbing properties in the μRB hydrogels. The compressive modulus of the hydrogel was increased up to 255 kPa post twenty-one days of MSC seeding. On the contrary, traditional bulk hydrogels show a compressive modulus of approximately 60–65 kPa.[47] Such a substantial increase in the mechanical properties is due to enhanced neocartilage formation within the microporous structure of μRB hydrogels. Another group attempted to reinforce the gelatin μRB hydrogel with chondroitin sulfate and observed an increased compressive modulus (355 kPa) when loaded with adipose-derived stem cells and neonatal chondrocytes. The authors hypothesized that adding chondroitin sulfate to the μRB hydrogel might have facilitated increased deposition of the ECM by the stem cells and chondrocytes.[166] Although these studies did not fully explored high aspect ratio μRB hydrogels for MIPs, reports suggest that they have excellent shear thinning properties,[130] making them a promising candidate for MIPs.

As the microporous structure of granular hydrogel can promote stem cell survival and their cell–cell communication required for chondrogenesis, similar approach was adopted by Fanyi Li *et al.* in which they introduced stem cell-laden gelatin norbornene (GelNB) – poly(ethylene glycol) dithiol microgels for intra-articular delivery to treat cartilage lesions.[65,167] The microgel system promoted chondrogenic differentiation of stem cells compared to the bulk hydrogels. Significant expression of TGF-β and collagen II was observed in the stem cell-encapsulated microgels. When injected in the subcutaneous region of the mouse model, it was observed that the cartilage matrix was homogenously distributed, and the cartilaginous tissue occupied the inter-microgel spaces analogous to the natural cartilage.[65] However, they did observe multiple cavities within the microgel system, possibly due to accelerated degradation by the matrix metalloproteinases. Also, type I collagen was significantly expressed along with type II, which suggests that a continuous and prolonged chondrogenic induction is required for regenerating native cartilage. To stabilize the granular hydrogel properties and improve the long-term maintenance of encapsulated stem cells, the same group utilized a 4-arm poly(ethylene glycol)-*N*-hydroxysuccinimide (NHS) crosslinker to induce covalent bonds in the gelatin/polyethylene glycol (PEG) microgel blocks to form microgel assembly and achieve chondrogenesis.[51] They functionalized gelatin with norbornene and PEG with thiol moieties to fabricate microgels with a pipette tip-based microfluidic process and subsequently crosslink the microgel droplets with blue light. This stem cell-loaded microgel assembly not only increased the expression of chondrogenic markers such as Sox9, Collagen II, and aggrecan but also increased the ECM production, as confirmed by alcian blue and safranin O staining. However, the degradation profile of this microgel assembly needs to be evaluated *in vivo* to check the efficacy of microgels for cartilage regeneration, as the inflammatory stress and protease enzymes can alter the rate of degradation of the microgel assembly.

Granular hydrogels can also be employed for the targeted delivery of cells at the disease site to provide a concentrated and localised treatment for tailoring cartilage regeneration.[168] The unique physical properties of such microporous hydrogels furnish a supportive microenvironment for the cells to proliferate and differentiate into a desired phenotype. Along the same line, Yu Zhu *et al.* leveraged materials such as hyaluronic acid, polyethylene glycol, and gelatin to fabricate granular hydrogel for the deployment of chondrocytes for cartilage regeneration.[96] The hydrogel microparticles were annealed with photocrosslinks to provide stability to the hydrogel structure. They observed enhanced hyaline cartilage matrix deposition and studied the role of AMP-activated protein kinase/glycolysis pathway in promoting the chondrogenic phenotype of the

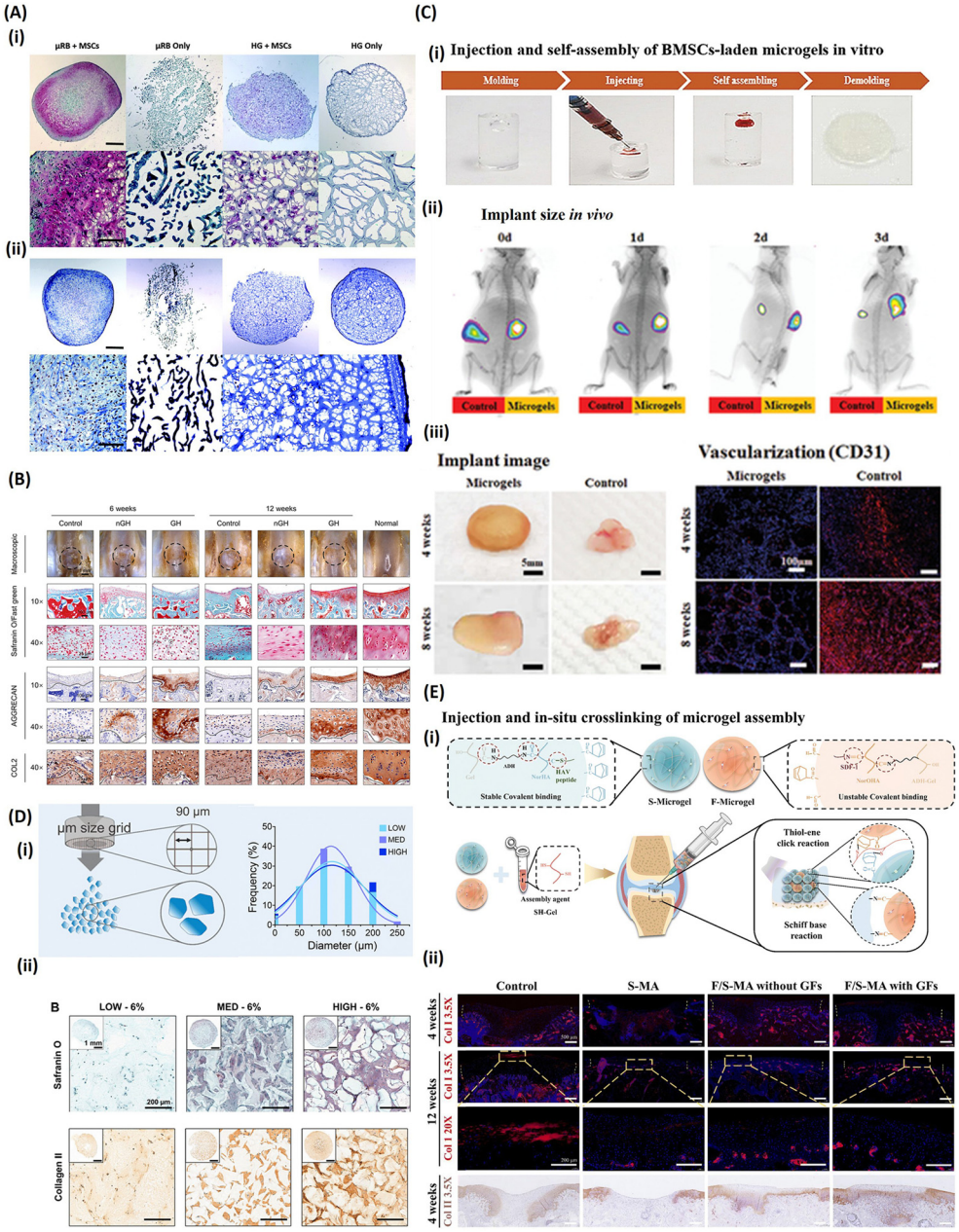


Fig. 8 Application of granular hydrogels as injectable hydrogel for cartilage tissue engineering. (A) μ-Ribbon based granular hydrogel assembly loaded with MSCs can promote efficient chondrogenesis. (i) Safranin-O staining depicting enhanced GAGs deposition in μ-ribbon granular hydrogels, (ii) trichrome staining showing similar trends with enhanced collagen deposition observed in granular gels, reproduced from ref. 143 with permission. Copyright©2018, Mary Ann Liebert, Inc. (B) Photo annealable granular hydrogel can be utilized to deliver chondrocytes to cartilage lesions and can promote hyaline cartilage regeneration. Histological examination depicting effectiveness of granular hydrogels in cell delivery over bulk hydrogels, reproduced from ref. 96 with permission. Copyright©2022, American Chemical Society. (C) Injectable granular hydrogel assembly developed from vinyl sulfonated hyaluronic acid (HA-VS) and thiolated gelatin (Gel-SH) can be used for cartilage regeneration through controlled neovascularization. (i) Injectability evaluation of granular cell loaded injectables, (ii) *in vivo* implantation of MSCs loaded granular injectable hypodermically into nude mice to evaluate the ectopic cartilage formation, (iii) granular assemblies demonstrated controlled vascularization and prevented hypertrophy over 8 weeks of implantation, leading to effective cartilage regeneration, reproduced from ref. 169 with permission. Copyright©2019, Wiley-VCH GmbH. (D) Zwitterionic granular hydrogel can be developed using mechanical fragmentation from bulk hydrogels and can be annealed enzymatically. (i) Microparticles of varied stiffness can be generated *via* extrusion of bulk TEDA hydrogels of varied stiffness, (ii) impact of varying particle stiffness and granular hydrogel porosity on chondrogenic potential of developed granular hydrogels. Authors demonstrated the superiority of porosity over stiffness in controlling the extent of chondrogenesis, reproduced from ref. 171 with permission. Copyright©2024, The Royal Society of Chemistry. (E) Heterogeneous granular assembly developed using fast and slow degrading microgels annealed *via* thiol–ene chemistry can allow efficient cartilage regeneration post-injection. (i) The schematic representation of generating hydrogel microparticles and corresponding interparticle annealing using thiol–ene chemistry, (ii) Col-I and Col-II deposition in cartilage lesion model illustrating efficient therapeutic potential of granular hydrogels post injection, reproduced from ref. 172 with permission. Copyright©2024, The Royal Society of Chemistry.

enclosed cells (Fig. 8B). Interestingly, a study by Qi Feng *et al.* demonstrated the self-assembly phenomenon in the microgel due to cell-to-cell connectivity when the cell grows rapidly over time on the surface of the microgel.[169] The microgel precursors used in this study were thiolated gelatin and vinyl-sulfonated hyaluronic acid. The bone mesenchymal stem cell-laden microgels were fabricated using droplet-based microfluidic techniques, and they showed excellent cell viability and proliferation. The *in vivo* implantation of this system revealed that the stem cell's interconnectivity led to microgel assembly without any additional crosslinking and prevented neovascularization and hypertrophy, thus mimicking the native cartilage (Fig. 8C). Furthermore, immunohistochemistry showed significant cartilage matrix deposition in the microgel implant. Although these studies successfully demonstrated the efficacy of stem cell encapsulated microgels for cartilage tissue engineering, they did not consider the innate immune response from the host, which can impact the stem cell survival. To address such concern, Ma *et al.* designed macrophage membrane-decorated microgels using physical crosslinking between gelatine and chondroitin sulfate with hydrogen and ionic interactions.[170] They overexpressed the matrix metalloproteinases using trypsin to hydrolyze gelatin and release chondroitin sulfate (66%) from the microgel in an inflammation-responsive manner, which was significantly less in the absence of trypsin (2%). The macrophage membrane-coated microgels can be localized at the inflammatory sites in an *in vivo* environment. In the osteoarthritis mice model, the microgels substantially decreased joint erosion, inhibited chondrocyte apoptosis, and promoted glycosaminoglycan deposition.

Further, zwitterionic granular hydrogels can be exploited to suppress innate immune response from the host due to their superior antifouling properties. The degree of crosslinking in zwitterionic granular hydrogels can directly affect the stiffness and porosities of the hydrogel, which can have an impact on chondrogenesis (Fig. 8D).[171] Using various concentrations of tetra (ethylene glycol) diacrylate (TEGDA), the zwitterionic Carboxybetaine acrylamide (CBAA) and tyramine acrylamide microgels were fabricated using the fragmentation method. The porosities in the granular hydrogel were found to be 40% in microgels crosslinked with high amounts of TEGDA. In contrast with the granular hydrogels prepared from fragmentation methods that have demonstrated poor control on the microgel porosities,[91] the authors were able to modulate porosities up to 40% by varying the cross-linking degree. The primary chondrocytes encapsulated within the granular hydrogel showed significant proliferation and spreading in the high TEGDA hydrogel with increased expression of Collagen II and Collagen I and secretion of GAG. The group was able to demonstrate that the stiffness was dominated by the void fraction in the granular hydrogel for chondrogenesis, which can help to improve cartilage regeneration. Zequ Lin *et al.* designed a heterogenous microgel assembly consisting of fast and slow degrading microgels to release stromal cell factor-1 (SDF-1) for stem cell homing and formation of micro-nest within the microgel assembly (Fig. 8E).[172] The fast-degrading microgels or the F-microgels were prepared using Schiff base chemistry, while the S-microgels (slow-degrading microgels) were prepared with an amidation reaction. Thiolated gelatin was used as an assembly agent and was triggered by light after intra-articular injection to initiate microgel assembly. SDF-1 and N-cadherin peptides, conjugated to the microgels, tailored stem cell homing and subsequent chondrogenesis, respectively. This heterogenous microgel assembly modified with peptides increased the expression of collagen2, Sox9, and aggrecan, suggesting chondrogenic differentiation of the stem cells. Nikolas Di Caprio *et al.* fabricated MSC and granular hydrogel composite to facilitate cartilage regeneration by cell-to-cell contact guidance and granular hydrogel stability.[99] After centrifugation of the spheroids and granular hydrogels separately, they were mixed in various ratios. They found that 20 : 80 and 35 : 65 spheroid to granular hydrogel ratios gave a stable structure due to increased interparticle crosslinking in the granular hydrogels. The ability of the granular hydrogel composite to promote chondrogenesis was confirmed by increased expression of collagen II, Sox9, and aggrecan, further promoting GAG deposition. The shear-thinning and self-healing properties of MSC spheroid and granular hydrogel composite will allow injectability at the target site through MIPs.

An attempt has been made to formulate a nano-in-microgel system by incorporating drug-loaded PLGA nanoparticles and further conjugating the microgel with cartilage-binding peptides for articular cartilage regeneration.[40] The microgel consisting of 4-arm polyethylene glycol maleimide served as a depot for the drug-loaded PLGA nanoparticle and released the model drug sustainably for up to sixteen days. Whereas, drug-loaded PLGA nanoparticles delivered without hydrogel depots fail to retain the drug at the disease site and are usually cleared from the joint space within a week.[173,174] The addition of peptides aided the binding of the microgel to the native cartilage and synoviocytes. Similar patterns were observed in the rat osteoarthritis model. The drug-loaded nanocomposite microgel had a longer retention time in the rat joint when compared to the free drug. This study demonstrates that microgels can be efficiently used for therapeutic delivery in the interarticular space. Another study used kartogenin-loaded cyclodextrin nanoparticles to prepare nanocomposite stem cell-laden microgels for cartilage regeneration.[175] The microgel assembly was achieved by dynamic covalent bonds between the dopamine-conjugated hyaluronic acid and phenylboronic acid groups. As discussed earlier, the assembly in the current microgel systems is more uniform and efficient than the microgel assembly obtained by cell–cell interactions. The kartogenin-loaded nanoparticles helped positively modulate the chondrogenesis of stem cells along with the inherent microporous structure of the microgel. The *in vivo* rabbit testing also showed promising results in terms of cartilage regeneration with this microgel system. Anna Puiggalí-Jou *et al.* employed ionic interactions to load positively charged growth factors into the granular hydrogel using negatively charged sulfated hyaluronic acid methacrylate (SHAMA).[176] Growth factor-

Table 3 Table summarizing the application of granular hydrogel as an injectable material for cartilage tissue engineering

Microgel design	Microgel fabrication technique	Jamming mechanism	Mechanical properties	Cyto- and biocompatibility	Key highlights	Ref.
Hyaluronic acid, gelati, and polyethylene glycol	Emulsion	Interparticle linkage (covalent interaction)	—	• >90% cytocompatibility with bone mesenchymal stem cells • Biocompatible with mice.	• Annealed by photocrosslinking • Promoted ECM deposition *in vitro* • Regeneration of hyaline-like cartilage in animal models	91
Gelatin norbornene and polyethylene glycol dithiol	Microfluidics	Interparticle linkage (click chemistry)	Elastic modulus 5.9 ± 0.5 kPa at Day 1 and 18.8 ± 1.2 kPa at 21 days of chondrogenesis	• >80% cytocompatibility with human adult articular chondrocytes (hACs), chondroprogenitor cells (hCCs) and mesenchymal stem cells (hMSCs) • Biocompatibile with mouse models	• Native articular cartilage morphology regenerated in microgels implanted in the mouse • The newly formed ECM was uniformly distributed across the implanted microgel	59
Zwitterionic carboxybetaine acrylamide (CBAA) and Zwitterionic sulfobetaine methacrylate (SBAA)	Fragmentation	Interparticle linkage (enzymatic)	Compressive modulus 4–6 kPa at Day 1 and 20–50 kPa at 21 days of chondrogenesis	• Cytocompatible with primary chondrocytes	• Demonstration of a strategy to develop zwitterionic granular hydrogels • The microporous hydrogel showed properties to promote chondrogenesis with increased porosity	81
Thiolated gelatin and vinyl sulfonated hyaluronic acid	Microfluidic	Interparticle linkage (*in situ* by cells)	~25 kPa compressive modulus of the microgel implanted *in vivo*.	• Cytocompatible with bone mesenchymal stem cells • Biocompatible with mice.	• Microgel assembly triggered by cell–cell interconnectivity • Ectopic cartilage regeneration observed in nude mice	163
Carboxybetaine acrylamide and tyramine acrylamide polymerized using TEGDA or GelMa	Fragmentation	Interparticle linkage (enzymatic)	Microgel stiffness 3 kPa Elastic modulus 1 kPa at Day 1 and ~35 kPa at 21 days of chondrogenesis	• Cytocompatible with primary human chondrocytes	• Inter-microgel crosslinking achieved by HRP/H_2O_2 crosslinking • The microgel porosities played a more prominent role in chondrogenesis than the stiffness	165
Norbornene-modified hyaluronic acid, norbornene-modified oxidized hyaluronic acid, hydrazide-modified gelatin and cysteine hydrochloride-modified gelatin	Emulsification	Interparticle linkage (thiol–ene chemistry)	Tensile strength 3.395 kPa (tissue adhesion strength of microgel tested on pig skin)	• Cytocompatible with bone mesenchymal stem cells • Biocompatible with New Zealand rabbits	• Heterogenous microgel assembly consisting of slow and fast degrading microgels • Release of stromal cell-derived factor 1 from fast degrading microgels recruited stem cells and HAV peptides in the slow degrading microgel induced chondrogenesis.	166
Norbornene-modified hyaluronic acid	Emulsification	Centrifugation	Compressive modulus <5 kPa at Day 1 and ~580 kPa at 56 Days of chondrogenesis	Cytocompatible with mesenchymal stromal cells	• An injectable granular composite formed Injectable MSC Spheroid • Dramatic cell–cell communication was achieved by this composite which favoured significant chondrogenesis. • Increased expression of collagen II, Sox9, aggrecan and increased GAG deposition	94

Table 3 *(Contd.)*

Microgel design	Microgel fabrication technique	Jamming mechanism	Mechanical properties	Cyto- and biocompatibility	Key highlights	Ref.
Hyaluronic acid methacrylate and gelatin methacrylate	Microfluidics	Interparticle linkage (click chemistry)	—	• Cytocompatible with bone mesenchymal stem cells • Biocompatible with New Zealand rabbits	• Kartogenin-loaded acetalated acrylate β-cyclodextrin nanoparticles were added to induce chondrogenesis • Jamming was achieved by dynamic bonds between dopamine and phenylboronic acid moieties on the surface of the microgel • The O'Driscoll score was 25.33 for the nanoparticle-reinforced microgel in rabbits	169
Hyaluronic acid methacrylate and sulfated hyaluronic acid methacrylate	Fragmentation	Interparticle linkage (enzymatic)	Elastic modulus 6.5 ± 0.8 kPa at Day 1 and 37 ± 8 kPa at 21 days of chonrogenesis	• 85% cytocompatibility with bone marrow mesenchymal stem cells	• Growth factor loaded sulfate hyaluronic acid methacrylate islands in the heterogenous microgel • Annealed with enzymatic crosslinking • Significant cell homing and subsequent chondrogenesis in bovine osteochondral explant	170

loaded SHAMA created micro islands within the HAMA granular hydrogel, forming a heterogeneous system annealed with enzymatic crosslinking. This led to localized production of collagen-II and enhanced cartilage regeneration. Another important clinical concept is to design injectable hydrogels with radiopacity for image-guided delivery and post-injection monitoring of the hydrogels to optimise the probability of treatment success in osteoarthritis. Radiopaque hydrogels allow the non-invasive monitoring of the injectable biomaterials, which have been shown to play an effective role in determining the efficacy of such hydrogels.[178] For example, Gullbrand and group[177] synthesized hyaluronic acid injectable granular hydrogels encapsulated with zirconium oxide nanoparticles for intervertebral disc regeneration.[178] The nanoparticles imparted radiopacity to the granular hydrogel, and this composite demonstrated promising results for intervertebral disc repair and large preclinical models. Such strategies can also be explored for knee cartilage regeneration.

Overall, this evidence strongly supports the possibility of a successful transition of granular hydrogels from lab to clinics and holds tremendous therapeutic potential for MIPs related to cartilage tissue repair and regeneration.[175,176] Although the research focusing on granular hydrogels for cartilage is in the early stage, significant *in vitro* and preclinical data suggest that the granular hydrogel system can effectively engineer and restore the structure and functions of cartilage. In the coming years, it is expected that the microgels will be tested and evaluated in large animal models for restoring the structural, biochemical, and functional properties of the native cartilage to boost the translation process. Key progresses made in the granular hydrogels for minimally invasive procedures for cartilage repair and regeneration has been highlighted in Table 3.

3.1.2 Granular hydrogel as bioinks for cartilage tissue engineering. When granular hydrogels are annealed to form a jammed state, they behave like yield stress solids, which allows them to remain in a solid state at rest and become fluidic upon sufficient stress. Such a stress-dependent material property is relevant for extrusion-based bioprinting, in which a biomaterial must undergo shear stress to be extruded from the nozzle and solidify rapidly to maintain the shape fidelity.[21,135] The physical interactions between the granular hydrogel microparticles responsible for the yield stress behaviour can endow unique rheological properties to the hydrogel without interfering with the molecular compositions of the materials. As a result, the cell viability is not compromised in the case of 3D bioprinting with granular hydrogel bioinks due to their unique shear-thinning behaviour compared with traditional bioinks. Furthermore, microporous structures and excellent pore interconnectivity in the granular hydrogels can help in effective cellular communication for cartilage regeneration.[179] This unique blend of printability and porosity makes them a more promising alternative bioinks for the extrusion-based 3D bioprinting of complex architectures, particularly for complete thickness cartilage defects.[62,164]

In this context, Xin *et al.* 3D bioprinted polyethylene glycol (PEG) microgels synthesised by the electrospraying tech-

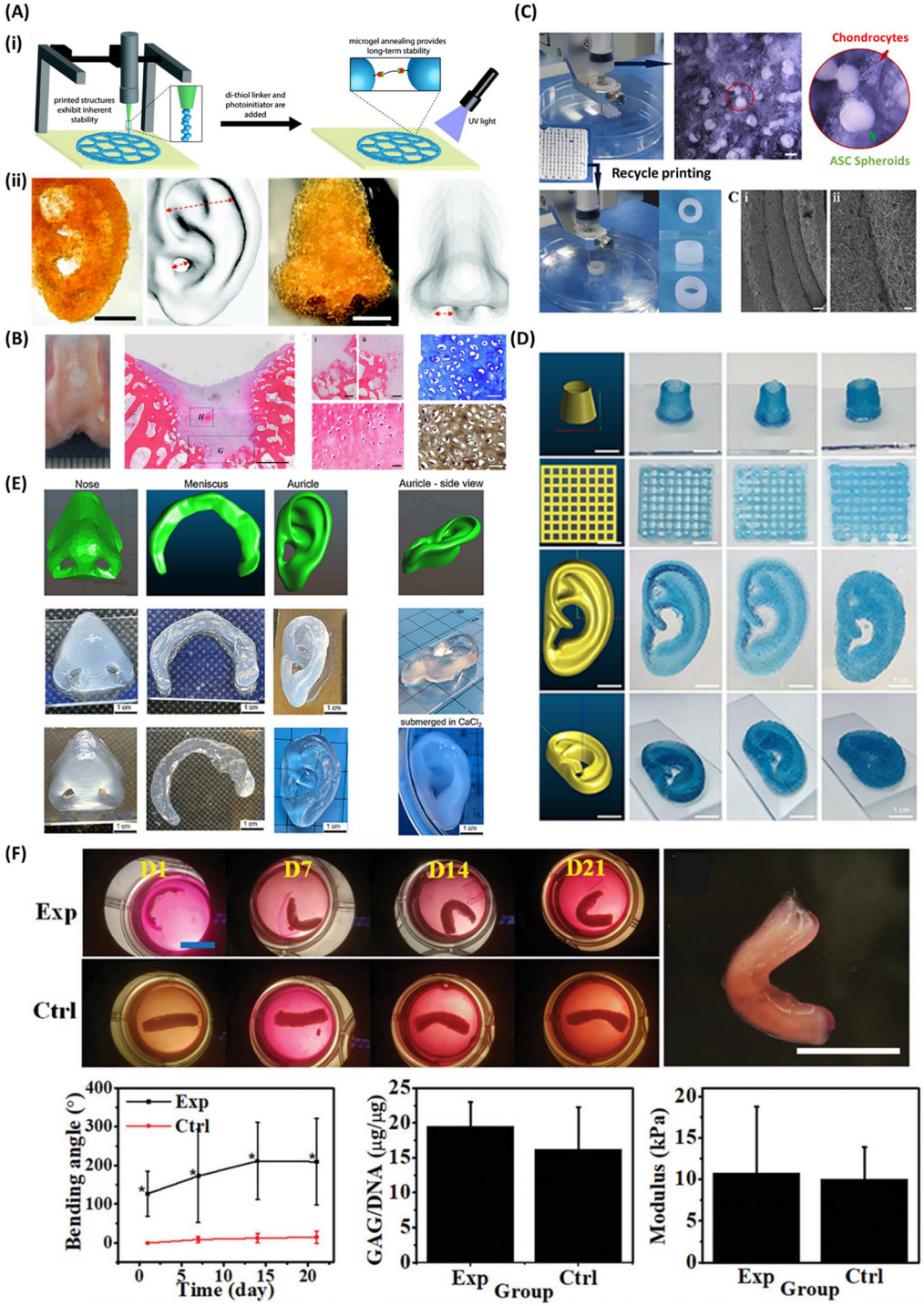


Fig. 9 Granular hydrogels can be utilized as bioinks for 3D bioprinting of patient-specific implants for large cartilage defects. (A) Photo-clickable PEG microgels utilization for the development of granular hydrogel bioinks. (i) Schematic representation of 3D bioprinting process with stabilization of structures post-biofabrication, (ii) printed cartilage mimicking geometries with high shape fidelity and resolution, reproduced from ref. 63 with permission. Copyright©2019, The Royal Society of Chemistry. (B) Zwitterionic-based granular hydrogel bioprinted constructs implanted at the site of cartilage damage can promote efficient neo-cartilage formation, reproduced from ref. 181 with permission. Copyright©2024, Wiley-VCH GmbH. (C) Host–guest interaction driven granular hydrogels coupled with chondrocytes and ASC spheroids can be utilized to bio print fully matured cartilage constructs, reproduced from ref. 20 with permission. Copyright©2022, IOP Publishing Ltd. (D) Hyaluronic acid-based granular bioinks showing excellent shear thinning properties and ability to create cartilage-mimicking tissue constructs with high resolutions that can be utilized as patient-specific grafts, reproduced from ref. 182 with permission. Copyright©2022, IOP Publishing Ltd. (E) Ionically annealable granular hydrogel bioinks demonstrating abilities for high-resolution 3D bioprinting, reproduced from ref. 183 with permission. Copyright©2024, IOP Publishing Ltd. (F) 4D bioprinted cartilage-like constructs showing enhanced chondrogenesis over 21 days of culture, reproduced from ref. 89 with permission. Copyright©2022, Wiley-VCH GmbH.

nique.[63] The authors employed click-chemistry to crosslink the microgels, and the 3D printed structures were further annealed by secondary crosslinking of the residual norbornene groups (Fig. 9Ai). They were able to print structures up to 2 cm in height without collapsing. Complex structures like the ear and nose were printed with high precision (~4% greater than the desired dimensions) using an 18-gauge nozzle (Fig. 9Aii). Decellularized extracellular matrix (dECM) can help in providing a suitable microenvironment for cellular functions, as it contains tissue-specific biomolecules and can serve as an excellent biomaterial for tissue regeneration.[180] Taking the bioactivity of dECM into consideration, Zhang *et al.* designed a granular bioink using a composite of dECM microparticles and gelatin methacrylate (GelMa) microspheres.[181] Polydopamine (PDA) was coated on dECM microparticles, and hyaluronic acid was added to the GelMa microspheres to lubricate the micro-interface friction. This granular bioink composition endowed superior printability, fidelity, and post-printing structural stability to the scaffolds. When implanted in the osteochondral defects of a rabbit, the granular ink scaffolds showed signs of neo-cartilage formations as compared to the blank group, which showed deposition of fibrous tissue in the defects. Significant expression of GAGs and collagen II was also observed in the experimental group (Fig. 9B). Another group exploited the granular hydrogel stem to produce stem cell spheroids within the hydrogel microparticle matrix.[20] Porous granular hydrogels were formed by β-cyclodextrin (β-CD) and poly(*N*-isopropylacrylamide) (PNIPAm) using the host–guest interactions. The microporous structure allowed the aggregation of stem cells, resulting in the formation of spheroids. Subsequently, chondrocytes and stem cell spheroids were encapsulated in granular hydrogel bioink, which exhibited outstanding shear-thinning and self-healing properties. The granular bioink was able to be printed in various shapes in a multilayer fashion, depicting the potential of this bioink for printing cartilage-like structures (Fig. 9C). M Wong and group utilised tyramine crosslinked hyaluronic acid granular hydrogel with varied microgel size to investigate the effect of hydrogel porosity on the secretion of ECM by the chondrocytes.[182] The granular hydrogels were prepared by extruding the bulk hydrogel through sieves with average aperture diameters of 40, 100, and 500 μm. The granular hydrogel demonstrated excellent injectability, printing precision, and ability to print complex cartilage structures (Fig. 9D). The group observed an inverse correlation between the pore interconnectivity and the aperture diameter. As a result, the granular hydrogel fragmented through 40 μm grids showed enhanced GAG deposition and tissue maturation following subcutaneous implantation in mice. However, collagen I expression was also seen in the microgel, indicating the microgels did not form fully mature cartilage. *In vivo* implantation also elicited a magnificent inflammatory response, especially in the microgels with a large particle size. This may hamper the tissue regeneration process. Granular hydrogels with immunomodulatory and chondrogenic properties may combat such problems *in vivo.* In another study, the same group fabricated microstrand using hyaluronic acid methacrylate, which randomly entangle with each other, imparting structural stability without any secondary crosslinks.[130] The hydrogel microstrands were compatible with chondrocytes and promoted chondrogenesis. Zwitterionic granular hydrogels have also been investigated as bioinks possessing outstanding rheological properties and the ability to print large and complex structures. Such granular bioinks are able to produce and reorganise cell spheroids after 3D bioprinting, showing a promise for *in vivo* tissue regeneration.[19] Furthermore, zwitterionic granular hydrogel's anti-fouling properties can effectively inhibit the foreign body response, aiding in rapid cartilage regeneration (Fig. 9E).[183]

4D printing is an emerging field that evolved from 3D printing, where the printed scaffold undergoes dynamic reconfiguration upon exposure to predetermined stimuli. 4D printing enables the construction of a smart system that can mimic the complex tissue hierarchy and utilize natural or *in vivo* stimuli to undergo dynamic transformations. 4D bioprinting of biocompatible materials to develop cell-laden constructs for cartilage regeneration can be of great significance.[184,185] 4D printing with bulk hydrogels poses numerous problems, such as poor print fidelity and resolution, toxicity due to fabrication techniques and degradation products, and restriction of cell–cell communications.[186,187] To overcome these hurdles, authors have attempted to use granular hydrogels for 4D bioprinting of cartilage constructs. For example, Ding *et al.* developed a single-component and heterogeneous microflake hydrogel using oxidised and methacrylated sodium alginate for 4D bioprinting.[90] The post-printing anisotropization due to photo-crosslinking induced the shape morphing of the constructs into predefined geometries. Mesenchymal stem cells encapsulated in the bioinks remained highly viable after 4D printing, and this cell-laden 4D printed scaffold promoted GAG deposition and tissue maturation after chondrogenic induction. In another study, the same group explored a 4D cell-condensate bioprinting approach to fabricate a bilayer structure to endow a shape transformation feature to a 3D printed cellular construct (Fig. 9F).[89] 4D bioprinting of granular hydrogels has fostered new ways to fabricate tissue constructs with reconfiguration dynamism to match the tissue transformation and remodelling processes during healing and regeneration.[188] Further preclinical studies should be directed to determine the potential use of such a technique for cartilage tissue regeneration. All this evidence confirms the potential of granular hydrogels as compelling bioinks for 3D and 4D bioprinting of tissue-mimetic constructs, which can integrate with host tissues and orchestrate rapid healing of the cartilage defects. Recent advancements made in the field of granular hydrogel bioprinting for cartilage tissue engineering have been highlighted in Table 4.

3.1.3 Clinical translation of granular hydrogel systems for cartilage tissue engineering. Cartilage tissue engineering approaches hold promising technology for treating chondral defects tailored with the emergence of biomaterials-based techniques and fabrication methods. MIPs are the clinically

Table 4 Table summarizing the applications of granular hydrogels as potential bioinks for 3D bioprinting for cartilage tissue engineering

Microgel design	Microgel fabrication technique	Microgel crosslinking strategy	3D printing technique	Mechanical properties	Cyto- and biocompatibility	Key highlights	Ref.
PEG-norbornene	Electrospraying	Photocrosslinking	Extrusion	Young's modulus ~40 kPa	• >90% cytocompatibility with human mesenchymal stem cells	• Microgel crosslinked *via* click chemistry • Bioprinting of complex geometries • Holds potential for cartilage regeneration	57
Decellularized extracellular matrix and gelatin methacrylate	Fragmentation	Photocrosslinking and Schiff-base chemistry	Extrusion	—	• ~90% cytocompatibility with chondrocytes • Biocompatible with rabbit models	• dECM microparticle coated with PDA • Excellent printability of granular inks with high dECM content • Robust cartilage regeneration	175
Acrylamide-β-cyclodextrin and poly(*N*-isopropylacrylamide)	Emulsion	Photocrosslinking	Extrusion	—	• Cytocompatible with rat adipose-derived stem cells • Biocompatible with mouse models	• Non-fouling matrix of granular hydrogel promoted spheroid formation. • Significant collagen II and aggrecan expression *in vivo*	14
Hyaluronic acid tyramine	Fragmentation	Enzyme-mediated crosslinking	Extrusion	Elastic modulus 7.5 ± 2.4 kPa at Day 1, 83.9 ± 19.7 kPa at Day 21, and 201.6 ± 8.9 kPa at 63 days of chondrogenesis	• >70% cytocompatibility with human auricular chondrocytes • Biocompatible with mouse models	• Annealing of the hydrogel microparticles achieved by secondary crosslinking • Enhanced synthesis of collagen II *in vivo*	176
Hyaluronic acid methacrylate	Fragmentation	Photocrosslinking	Extrusion	Compressive modulus 2.7 ± 0.3 kPa at Day1, 212 ± 83.7 kPa at Day 21, and 780.2 ± 218.4 kPa at 42 days of chondrogenesis	• ~90% cytocompatibility with chondrocytes and myoblasts	• Porous and entangled microstructure of the granular hydrogel bioink • Excellent printability and anisotropicity. • Enhanced deposition of cartilaginous matrix.	157
Poly(sulfobetaine methacrylate-co-poly(ethylene glycol) diacrylate)	Emulsion	Photocrosslinking	Extrusion	Compressive modulus 2.67 ± 0.92 MPa (neo-cartilage formed after implantation in the osteochondral defects of rabbits)	• Cytocompatible with adipose-derived stem cells • Biocompatible with rabbit models	• Outstanding shear-thinning and self-healing properties • Composite granular hydrogel bioink formed by *in situ* polymerization • Bioink capable of producing stem cell spheroids	13
Carboxybetaine acrylamide and sulfobetaine methacrylate with alginate methacrylate crosslinker	Fragmentation	Photocrosslinking	Extrusion	Compressive modulus ~20 kPa at Day 1, 119 ± 28 kPa at Day 21 of chondrogenesis	• ~90% cytocompatibility with human articular chondrocyte	• The zwitterionic microgel had tunable porosity and excellent printability • Promoted cartilage ECM deposition • Scaffolds showed a non-immunogenic and anti-inflammatory response	178

Table 4 *(Contd.)*

Microgel design	Microgel fabrication technique	Microgel crosslinking strategy	3D printing technique	Mechanical properties	Cyto- and biocompatibility	Key highlights	Ref.
Oxidised and methacrylate alginate and gelatin methacrylate	Fragmentation	Ionic and photo-crosslinking	Extrusion (4D cell-condensate)	Elastic modulus ~10 kPa	• Cytocompatible with human mesenchymal stem cells	• Shape morphing achieved by bioprinting a bilayer gradient structure • The bilayer consisted of a gradient crosslinked 3D printed actuation layer • The bioprinted second layer formed condensate on the actuation layer	84
Norbornene modified hyaluronic acid	Emulsion	Photo crosslinking	Extrusion (4D bioprinting)	—	• Cytocompatibility with MSC spheroids	• Enhanced chondrogenesis after 28 days • Shape deformation into cartilage-like self-standing structures after 28 days	183

most preferred methods for cartilage repair and regeneration, drawing considerable focus on injectable hydrogels. Injectable hydrogels have also been widely used and investigated in other tissue engineering sectors, such as bone, wound healing, cardiac tissue engineering, *etc*. Around 30 clinical studies involve injectable hydrogels for tissue engineering applications.[189] It is worthwhile to mention that, though bulk hydrogels have excellent biocompatibility, degradation profile, drug or growth factor loading capacity, and ability to fit into irregular defects, they have not yet achieved good clinical outcomes in terms of cartilage regeneration, and there remains a vast space for improvement. Granular hydrogels can address this limitation due to their versatility and numerous advantages, such as high surface-to-volume ratio, microporous structure, shear thinning, and self-healing properties.[70,94] As mentioned earlier, one of the most prominent attributes exhibited by granular hydrogels is injectability, which allows the hydrogel to be delivered with MIP, leading to less pain and preventing the chances of secondary infections.[40] Currently, granular hydrogels have been only studied in preclinical animal models as the field has recently emerged but holds the potential to be used in clinics in the future.[190] Though granular hydrogels are proving to be a better therapeutic strategy for cartilage regeneration, as demonstrated in the *in vitro* and preclinical studies, there remain some significant barriers that must be bridged before translating granular hydrogels into clinics.[191] Large-scale fabrication methods need to be evolved to meet clinical needs while maintaining quality standards, sterility, cost-effectiveness, and safety. Batch-to-batch uniformity of the granular hydrogel system is of utmost importance. It can be achieved by a few techniques like droplet-based microfluidics,[110,163].[192] Furthermore, to be commercialized into the markets, such a product intended for tissue engineering applications must go through regulatory pathways, which may vary in different countries. Also, the production of granular hydrogels should be in accordance with the ISO standards and Good Manufacturing Practices (GMP).[163] Another concern is the limitations of the choice of biomaterials for granular hydrogel fabrication, especially for cartilage tissue engineering, as not many biomaterials have been approved by the FDA. To summarize, despite these barriers, granular hydrogels represent a next-generation therapy with dynamic properties for cartilage tissue regeneration. Cell-laden and/or growth factor-loaded hydrogels are also emerging with immense potential to treat cartilage defects and injuries. Ensuring regulatory and safety compliance with additional studies of granular hydrogels in large preclinical models and clinical trials for their safety and efficacy can aid in translating this technology for cartilage-related therapies.

4 Conclusion and future perspective

Granular hydrogels have emerged as a transformative class of biomaterials in the field of cartilage tissue engineering, offering a compelling alternative to traditional bulk hydrogels.

Their intrinsic micro-porous architecture, formed through the assembly of hydrogel microparticles, enables superior control over mechanical and biochemical properties. This microstructural advantage supports enhanced cellular infiltration, nutrient diffusion, and overall tissue integration—critical factors for the successful regeneration of avascular tissues such as cartilage. Significant strides have been made in developing injectable granular hydrogels with tunable properties, allowing for minimally invasive delivery and *in situ* formation. Notably, the development of multifunctional granular systems capable of integrating biochemical cues, cells, or bioactive agents has significantly improved their regenerative potential. These systems can be finely engineered to mimic the native extracellular matrix (ECM), further supporting chondrogenesis and tissue repair.

Despite these promising advancements, key challenges must be addressed to facilitate clinical translation. Scaling up the production of granular hydrogels remains a technical bottleneck. Although recent innovations in high-throughput droplet generation have improved microgel synthesis, post-processing steps often slow down the overall fabrication workflow. Additionally, translating these methods to a good manufacturing practice (GMP) framework remains a critical next step for clinical approval and widespread use. Future research should also focus on the exploration of alternative biomaterials, particularly decellularized ECM and cartilage-derived extracts. These components hold promise for creating more biologically relevant hydrogel systems that closely emulate the native cartilage microenvironment. Such advancements could lead to more effective and durable cartilage repair strategies.

Furthermore, while current preclinical studies predominantly utilize acellular formulations, the incorporation of living cells—such as mesenchymal stem cells (MSCs), chondrocytes, or engineered cell types—can substantially enhance regenerative outcomes. Coupling granular hydrogels with therapeutic agents or growth factors could also provide dynamic, on-demand responses to the injured environment. However, these strategies must consider potential host immune responses and ensure the survival and function of encapsulated cells post-implantation. The integration of advanced 3D biofabrication techniques, including robotic arm-based printing of granular hydrogels, opens exciting possibilities for intraoperative applications. Such technologies could enable patient-specific scaffold design and real-time cartilage repair, bridging the gap between bench-top development and bedside implementation.

In summary, granular hydrogels represent a promising frontier in cartilage tissue engineering, offering unparalleled flexibility, bioactivity, and minimally invasive delivery. With continued innovation in materials science, manufacturing technologies, and biological integration, these systems have the potential to redefine therapeutic strategies for cartilage repair. By addressing current translational challenges and leveraging emerging technologies, granular hydrogels are poised to become a cornerstone in the future of regenerative medicine.

Author's contribution

Akshat Joshi: conceptualization (lead), supervision (equal), writing – original draft (lead), writing – review & editing (lead). Akhilesh Agrawal: conceptualization (supporting), writing – original draft (equal), writing – review & editing (equal). Saswat Choudhury: writing – original draft (supporting), writing – review & editing (supporting). Subha Narayana Rath: conceptualization (equal), supervision (lead), writing – original draft (supporting), writing – review & editing (supporting). Akshay Joshi: writing – original draft (supporting), writing – review & editing (supporting). Kushal Taori: writing – original draft (supporting), writing – review & editing (supporting). Savadamoorthi Kamatchi Subramani: writing – original draft (supporting), writing – review & editing (supporting). Sabari Murugesan: writing – original draft (supporting), writing – review & editing (supporting). Ujjayan Majumdar: writing – original draft (supporting), writing – review & editing (supporting). Ji-hoo Lee: writing – original draft (supporting), writing – review & editing (supporting). Suk-Jung Oh: conceptualization (equal), supervision (supporting), writing – original draft (supporting), writing – review & editing (supporting).

Conflicts of interest

The authors have no conflict of interest to declare.

Data availability

No new data were generated or analyzed in this study. All data cited are available in the referenced publications.

Acknowledgements

The authors acknowledge the Datta Meghe Institute of Higher Education and Research, NewGen IEDC, for funding. Akhilesh Agrawal thanks the Nurturing Clinical Scientist Fellowship by the Indian Council of Medical Research (ICMR). Akshay Joshi acknowledges financial support from the American Heart Association (Award number: 24POST1241463). Akshat Joshi acknowledges Ecoworld Pharmaceuticals for their insights and resources.

References

1 T. Stampoultzis, P. Karami and D. P. Pioletti, Thoughts on cartilage tissue engineering: A 21st century perspective, *Curr. Res. Transl. Med.*, 2021, **69**(3), 103299.
2 W. Wei and H. Dai, Articular cartilage and osteochondral tissue engineering techniques: Recent advances and challenges, *Bioact. Mater.*, 2021, **6**(12), 4830–4855.

3 X. Li, S. Sheng, G. Li, Y. Hu, F. Zhou, Z. Geng and J. Su, Research progress in hydrogels for cartilage organoids, *Adv. Healthcare Mater.*, 2024, **13**(22), 2400431.
4 X. Guo, Y. Ma, Y. Min, J. Sun, X. Shi, G. Gao, L. Sun and J. Wang, Progress and prospect of technical and regulatory challenges on tissue-engineered cartilage as therapeutic combination product, *Bioact. Mater.*, 2023, **20**, 501–518.
5 A. Trengove, C. Di Bella and A. J. O'Connor, The challenge of cartilage integration: understanding a major barrier to chondral repair, *Tissue Eng., Part B*, 2022, **28**(1), 114–128.
6 O. Kopinski-Grünwald, O. Guillaume, T. Ferner, B. Schädl and A. Ovsianikov, Scaffolded spheroids as building blocks for bottom-up cartilage tissue engineering show enhanced bioassembly dynamics, *Acta Biomater.*, 2024, **174**, 163–176.
7 H.-E. Shim, Y.-J. Kim, K. H. Park, H. Park, K. M. Huh and S.-W. Kang, Enhancing cartilage regeneration through spheroid culture and hyaluronic acid microparticles: A promising approach for tissue engineering, *Carbohydr. Polym.*, 2024, **328**, 121734.
8 A. T. Lam, S. Reuveny and S. K.-W. Oh, Human mesenchymal stem cell therapy for cartilage repair: Review on isolation, expansion, and constructs, *Stem Cell Res.*, 2020, **44**, 101738.
9 K. M. Hubka, R. L. Dahlin, V. V. Meretoja, F. K. Kasper and A. G. Mikos, Enhancing chondrogenic phenotype for cartilage tissue engineering: monoculture and coculture of articular chondrocytes and mesenchymal stem cells, *Tissue Eng., Part B*, 2014, **20**(6), 641–654.
10 S. L. Vega, M. Y. Kwon and J. A. Burdick, Recent advances in hydrogels for cartilage tissue engineering, *Eur. Cells Mater.*, 2017, **33**, 59.
11 F. Hashemi-Afzal, H. Fallahi, F. Bagheri, M. N. Collins, M. B. Eslaminejad and H. Seitz, Advancements in hydrogel design for articular cartilage regeneration: A comprehensive review, *Bioact. Mater.*, 2025, **43**, 1–31.
12 N. Petitjean, P. Canadas, P. Royer, D. Noël and S. Le Floc'h, Cartilage biomechanics: From the basic facts to the challenges of tissue engineering, *J. Biomed. Mater. Res., Part A*, 2023, **111**(7), 1067–1089.
13 I. L. Kim, R. L. Mauck and J. A. Burdick, Hydrogel design for cartilage tissue engineering: a case study with hyaluronic acid, *Biomaterials*, 2011, **32**(34), 8771–8782.
14 L. Sun, Y. Xu, Y. Han, J. Cui, Z. Jing, D. Li, J. Liu, C. Xiao, D. Li and B. Cai, Collagen–Based Hydrogels for Cartilage Regeneration, *Orthop. Surg.*, 2023, **15**(12), 3026–3045.
15 C. Shen, Z. Zhou, R. Li, S. Yang, D. Zhou, F. Zhou, Z. Geng and J. Su, Silk fibroin-based hydrogels for cartilage organoids in osteoarthritis treatment, *Theranostics*, 2025, **15**(2), 560.
16 R. Zhang, S. J. Chang, Y. Jing, L. Wang, C.-J. Chen and J.-T. Liu, Application of chitosan with different molecular weights in cartilage tissue engineering, *Carbohydr. Polym.*, 2023, **314**, 120890.
17 Z. Ma, Y. Wu, G. Li, J. Liu, Z. Geng and J. Su, Extracellular vesicles-loaded DNA hydrogels: a promising candidate for cartilage organoids engineering, *Chem. Eng. J.*, 2023, **477**, 147146.
18 T. H. Qazi and J. A. Burdick, Granular hydrogels for endogenous tissue repair, *Biomater. Biosyst.*, 2021, **1**, 100008.
19 J. Zhang, W. Xin, Y. Qin, Y. Hong, Z. Xiahou, K. Zhang, P. Fu and J. Yin, "All-in-one" zwitterionic granular hydrogel bioink for stem cell spheroids production and 3D bioprinting, *Chem. Eng. J.*, 2022, **430**, 132713.
20 L. Zhang, H. Tang, Z. Xiahou, J. Zhang, Y. She, K. Zhang, X. Hu, J. Yin and C. Chen, Solid multifunctional granular bioink for constructing chondroid basing on stem cell spheroids and chondrocytes, *Biofabrication*, 2022, **14**(3), 035003.
21 C. Tuftee, E. Alsberg, I. T. Ozbolat and M. Rizwan, Emerging granular hydrogel bioinks to improve biological function in bioprinted constructs, *Trends Biotechnol.*, 2024, **42**(3), 339–352.
22 W. Wei, Y. Ma, X. Yao, W. Zhou, X. Wang, C. Li, J. Lin, Q. He, S. Leptihn and H. Ouyang, Advanced hydrogels for the repair of cartilage defects and regeneration, *Bioact. Mater.*, 2021, **6**(4), 998–1011.
23 S. E. Doyle, F. Snow, S. Duchi, C. D. O'Connell, C. Onofrillo, C. Di Bella and E. Pirogova, 3D printed multiphasic scaffolds for osteochondral repair: challenges and opportunities, *Int. J. Mol. Sci.*, 2021, **22**(22), 12420.
24 X. Lin, Y. Zhang, J. Li, B. G. Oliver, B. Wang, H. Li, K.-T. Yong and J. J. Li, Biomimetic multizonal scaffolds for the reconstruction of zonal articular cartilage in chondral and osteochondral defects, *Bioact. Mater.*, 2025, **43**, 510–549.
25 A. M. Seitz, D. Warnecke and L. Dürselen, Cartilage biomechanics, in *Human Orthopaedic Biomechanics*, Elsevier, 2022, pp. 151–176.
26 L. Fu, Z. Yang, C. Gao, H. Li, Z. Yuan, F. Wang, X. Sui, S. Liu and Q. Guo, Advances and prospects in biomimetic multilayered scaffolds for articular cartilage regeneration, *Regener. Biomater.*, 2020, **7**(6), 527–542.
27 W. Wang, R. Ye, W. Xie, Y. Zhang, S. An, Y. Li and Y. Zhou, Roles of the calcified cartilage layer and its tissue engineering reconstruction in osteoarthritis treatment, *Front. Bioeng. Biotechnol.*, 2022, **10**, 911281.
28 J. Antons, M. G. M. Marascio, J. Nohava, R. Martin, L. Applegate, P. Bourban and D. Pioletti, Zone-dependent mechanical properties of human articular cartilage obtained by indentation measurements, *J. Mater. Sci.: Mater. Med.*, 2018, **29**, 1–8.
29 A. Atwal, T. P. Dale, M. Snow, N. R. Forsyth and P. Davoodi, Injectable hydrogels: An emerging therapeutic strategy for cartilage regeneration, *Adv. Colloid Interface Sci.*, 2023, **321**, 103030.
30 J. Martel-Pelletier, A. J. Barr, F. M. Cicuttini, P. G. Conaghan, C. Cooper, M. B. Goldring, S. R. Goldring, G. Jones, A. J. Teichtahl and J.-P. Pelletier, Osteoarthritis, *Nat. Rev. Dis. Primers*, 2016, **2**(1), 16072.
31 Y. Zhang and J. M. Jordan, Epidemiology of osteoarthritis, *Rheum. Dis. Clin. N. Am*, 2008, **34**(3), 515–529.

32 S. Akizuki, V. C. Mow, F. Muller, J. C. Pita and D. S. Howell, Tensile properties of human knee joint cartilage. II. Correlations between weight bearing and tissue pathology and the kinetics of swelling, *J. Orthop. Res.*, 1987, **5**(2), 173–186.
33 S. R. Goldring and M. B. Goldring, Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage–bone crosstalk, *Nat. Rev. Rheumatol.*, 2016, **12**(11), 632–644.
34 B. J. De Lange-Brokaar, A. Ioan-Facsinay, G. J. Van Osch, A.-M. Zuurmond, J. Schoones, R. E. Toes, T. W. Huizinga and M. Kloppenburg, Synovial inflammation, immune cells and their cytokines in osteoarthritis: a review, *Osteoarthr. Cartil.*, 2012, **20**(12), 1484–1499.
35 F. H. Silver and A. I. Glasgold, Cartilage wound healing: an overview, *Otolaryngol. Clin. N. Am.*, 1995, **28**(5), 847–864.
36 M. Chen, Z. Jiang, X. Zou, X. You, Z. Cai and J. Huang, Advancements in tissue engineering for articular cartilage regeneration, *Heliyon*, 2024, **10**(3), e25400.
37 Y. Liu, K. M. Shah and J. Luo, Strategies for articular cartilage repair and regeneration, *Front. Bioeng. Biotechnol.*, 2021, **9**, 770655.
38 H. J. Faust, S. D. Sommerfeld, S. Rathod, A. Rittenbach, S. R. Banerjee, B. M. Tsui, M. Pomper, M. L. Amzel, A. Singh and J. H. Elisseeff, A hyaluronic acid binding peptide-polymer system for treating osteoarthritis, *Biomaterials*, 2018, **183**, 93–101.
39 T. P. Nguyen, F. Li, S. Shrestha, R. S. Tuan, H. Thissen, J. S. Forsythe and J. E. Frith, Cell-laden injectable microgels: Current status and future prospects for cartilage regeneration, *Biomaterials*, 2021, **279**, 121214.
40 L. M. Mancipe Castro, A. Sequeira, A. J. García and R. E. Guldberg, Articular cartilage-and synoviocyte-binding poly (ethylene glycol) nanocomposite microgels as intra-articular drug delivery vehicles for the treatment of osteoarthritis, *ACS Biomater. Sci. Eng.*, 2020, **6**(9), 5084–5095.
41 N. A. Householder, A. Raghuram, K. Agyare, S. Thipaphay and M. Zumwalt, A review of recent innovations in cartilage regeneration strategies for the treatment of primary osteoarthritis of the knee: Intra-articular injections, *Orthop. J. Sports Med.*, 2023, **11**(4), 23259671231155950.
42 M. Liu, X. Zeng, C. Ma, H. Yi, Z. Ali, X. Mou, S. Li, Y. Deng and N. He, Injectable hydrogels for cartilage and bone tissue engineering, *Bone Res.*, 2017, **5**(1), 1–20.
43 T. H. Qazi, D. J. Mooney, G. N. Duda and S. Geissler, Biomaterials that promote cell-cell interactions enhance the paracrine function of MSCs, *Biomaterials*, 2017, **140**, 103–114.
44 A. á. DeLise, L. Fischer and R. Tuan, Cellular interactions and signaling in cartilage development, *Osteoarthr. Cartil.*, 2000, **8**(5), 309–334.
45 H. Cao, L. Duan, Y. Zhang, J. Cao and K. Zhang, Current hydrogel advances in physicochemical and biological response-driven biomedical application diversity, *Signal Transduction Targeted Ther.*, 2021, **6**(1), 426.
46 G. Ying, N. Jiang, C. Parra-Cantu, G. Tang, J. Zhang, H. Wang, S. Chen, N. P. Huang, J. Xie and Y. S. Zhang, Bioprinted injectable hierarchically porous gelatin methacryloyl hydrogel constructs with shape–memory properties, *Adv. Funct. Mater.*, 2020, **30**(46), 2003740.
47 H. Li, K. S. Iyer, L. Bao, J. Zhai and J. J. Li, Advances in the development of granular microporous injectable hydrogels with non–spherical microgels and their applications in tissue regeneration, *Adv. Healthcare Mater.*, 2024, **13**(25), 2301597.
48 Q. Feng, D. Li, Q. Li, X. Cao and H. Dong, Microgel assembly: Fabrication, characteristics and application in tissue engineering and regenerative medicine, *Bioact. Mater.*, 2022, **9**, 105–119.
49 L. K. Meena, H. Rather, D. Kedaria and R. Vasita, Polymeric microgels for bone tissue engineering applications–a review, *Int. J. Polym. Mater. Polym. Biomater.*, 2020, **69**(6), 381–397.
50 A. C. Daly, L. Riley, T. Segura and J. A. Burdick, Hydrogel microparticles for biomedical applications, *Nat. Rev. Mater.*, 2020, **5**(1), 20–43.
51 F. Li, V. X. Truong, P. Fisch, C. Levinson, V. Glattauer, M. Zenobi-Wong, H. Thissen, J. S. Forsythe and J. E. Frith, Cartilage tissue formation through assembly of microgels containing mesenchymal stem cells, *Acta Biomater.*, 2018, **77**, 48–62.
52 E. L. Daley, R. M. Coleman and J. P. Stegemann, Biomimetic microbeads containing a chondroitin sulfate/chitosan polyelectrolyte complex for cell-based cartilage therapy, *J. Mater. Chem. B*, 2015, **3**(40), 7920–7929.
53 Q. Liang, Y. Ma, X. Yao and W. Wei, Advanced 3D-printing bioinks for articular cartilage repair, *Int. J. Bioprint.*, 2022, **8**(3), 511.
54 Y. Gu, Y. Zou, Y. Huang, R. Liang, Y. Wu, Y. Hu, Y. Hong, X. Zhang, Y.-C. Toh and H. Ouyang, 3D-printed biomimetic scaffolds with precisely controlled and tunable structures guide cell migration and promote regeneration of osteochondral defect, *Biofabrication*, 2023, **16**(1), 015003.
55 R. Jiao, X. Lin, J. Wang, C. Zhu, J. Hu, H. Gao and K. Zhang, 3D-printed constructs deliver bioactive cargos to expedite cartilage regeneration, *J. Pharm. Anal.*, 2023, **4**(12), 100925.
56 W. Cheng, J. Zhang, J. Liu and Z. Yu, Granular hydrogels for 3D bioprinting applications, *View*, 2020, **1**(3), 20200060.
57 A. R. Spencer, E. Shirzaei Sani, J. R. Soucy, C. C. Corbet, A. Primbetova, R. A. Koppes and N. Annabi, Bioprinting of a cell-laden conductive hydrogel composite, *ACS Appl. Mater. Interfaces*, 2019, **11**(34), 30518–30533.
58 D. Chimene, R. Kaunas and A. K. Gaharwar, Hydrogel bioink reinforcement for additive manufacturing: a focused review of emerging strategies, *Adv. Mater.*, 2020, **32**(1), 1902026.
59 K. S. Iyer, L. Bao, J. Zhai, A. Jayachandran, R. Luwor, J. J. Li and H. Li, Microgel-based bioink for extrusion-based 3D bioprinting and its applications in tissue engineering, *Bioact. Mater.*, 2025, **48**, 273–293.

60 K. A. Deo, K. A. Singh, C. W. Peak, D. L. Alge and A. K. Gaharwar, Bioprinting 101: design, fabrication, and evaluation of cell-laden 3D bioprinted scaffolds, *Tissue Eng., Part A*, 2020, **26**(5–6), 318–338.
61 R. Levato, T. Jungst, R. G. Scheuring, T. Blunk, J. Groll and J. Malda, From shape to function: the next step in bioprinting, *Adv. Mater.*, 2020, **32**(12), 1906423.
62 C. B. Highley, K. H. Song, A. C. Daly and J. A. Burdick, Jammed microgel inks for 3D printing applications, *Adv. Sci.*, 2019, **6**(1), 1801076.
63 S. Xin, D. Chimene, J. E. Garza, A. K. Gaharwar and D. L. Alge, Clickable PEG hydrogel microspheres as building blocks for 3D bioprinting, *Biomater. Sci.*, 2019, **7**(3), 1179–1187.
64 Y. Wang, X. Yuan, K. Yu, H. Meng, Y. Zheng, J. Peng, S. Lu, X. Liu, Y. Xie and K. Qiao, Fabrication of nanofibrous microcarriers mimicking extracellular matrix for functional microtissue formation and cartilage regeneration, *Biomaterials*, 2018, **171**, 118–132.
65 F. Li, C. Levinson, V. X. Truong, L. A. Laurent-Applegate, K. Maniura-Weber, H. Thissen, J. S. Forsythe, M. Zenobi-Wong and J. E. Frith, Microencapsulation improves chondrogenesis in vitro and cartilaginous matrix stability in vivo compared to bulk encapsulation, *Biomater. Sci.*, 2020, **8**(6), 1711–1725.
66 Z. Luo, J. Pan, Y. Sun, S. Zhang, Y. Yang, H. Liu, Y. Li, X. Xu, Y. Sui and S. Wei, Injectable 3D Porous Micro–Scaffolds with a Bio–Engine for Cell Transplantation and Tissue Regeneration, *Adv. Funct. Mater.*, 2018, **28**(41), 1804335.
67 A. Weizel, T. Distler, D. Schneidereit, O. Friedrich, L. Bräuer, F. Paulsen, R. Detsch, A. Boccaccini, S. Budday and H. Seitz, Complex mechanical behavior of human articular cartilage and hydrogels for cartilage repair, *Acta Biomater.*, 2020, **118**, 113–128.
68 K. Xue, X. Wang, P. W. Yong, D. J. Young, Y. L. Wu, Z. Li and X. J. Loh, Hydrogels as emerging materials for translational biomedicine, *Adv. Ther.*, 2019, **2**(1), 1800088.
69 X. Tong and F. Yang, Recent progress in developing injectable matrices for enhancing cell delivery and tissue regeneration, *Adv. Healthcare Mater.*, 2018, **7**(7), 1701065.
70 J. E. Mealy, J. J. Chung, H. H. Jeong, D. Issadore, D. Lee, P. Atluri and J. A. Burdick, Injectable granular hydrogels with multifunctional properties for biomedical applications, *Adv. Mater.*, 2018, **30**(20), 1705912.
71 E. Bakaic, N. M. Smeets and T. Hoare, Injectable hydrogels based on poly (ethylene glycol) and derivatives as functional biomaterials, *RSC Adv.*, 2015, **5**(45), 35469–35486.
72 V. K. Garripelli, J.-K. Kim, R. Namgung, W. J. Kim, M. A. Repka and S. Jo, A novel thermosensitive polymer with pH-dependent degradation for drug delivery, *Acta Biomater.*, 2010, **6**(2), 477–485.
73 T. Kamperman, M. Karperien, S. Le Gac and J. Leijten, Single-cell microgels: technology, challenges, and applications, *Trends Biotechnol.*, 2018, **36**(8), 850–865.
74 D. Seliktar, Designing cell-compatible hydrogels for biomedical applications, *Science*, 2012, **336**(6085), 1124–1128.
75 A. S. Mao, J.-W. Shin, S. Utech, H. Wang, O. Uzun, W. Li, M. Cooper, Y. Hu, L. Zhang and D. A. Weitz, Deterministic encapsulation of single cells in thin tunable microgels for niche modelling and therapeutic delivery, *Nat. Mater.*, 2017, **16**(2), 236–243.
76 L. Riley, L. Schirmer and T. Segura, Granular hydrogels: emergent properties of jammed hydrogel microparticles and their applications in tissue repair and regeneration, *Curr. Opin. Biotechnol.*, 2019, **60**, 1–8.
77 R. Shaik, J. Brown, J. Xu, R. Lamichhane, Y. Wang, Y. Hong and G. Zhang, Cardiac Matrix-Derived Granular Hydrogel Enhances Cell Function in 3D Culture, *ACS Appl. Mater. Interfaces*, 2024, **16**(43), 58346–58356.
78 M. Asadikorayem, P. Weber, F. Surman, A. Puiggalí-Jou and M. Zenobi-Wong, Foreign body immune response to zwitterionic and hyaluronic acid granular hydrogels made with mechanical fragmentation, *Adv. Healthcare Mater.*, 2025, **14**(2), 2402890.
79 V. G. Muir, M. E. Prendergast and J. A. Burdick, Fragmenting bulk hydrogels and processing into granular hydrogels for biomedical applications, *J. Visualized Exp.*, 2022, **17**(183), e63867.
80 G. I. Tanner, L. Schiltz, N. Narra, M. L. Figueiredo and T. H. Qazi, Granular hydrogels improve myogenic invasion and repair after volumetric muscle loss, *Adv. Healthcare Mater.*, 2024, **13**(25), 2303576.
81 T. H. Qazi, V. G. Muir and J. A. Burdick, Methods to characterize granular hydrogel rheological properties, porosity, and cell invasion, *ACS Biomater. Sci. Eng.*, 2022, **8**(4), 1427–1442.
82 A. R. Anderson, E. L. Caston, L. Riley, L. Nguyen, D. Ntekoumes, S. Gerecht and T. Segura, Engineering the Microstructure and Spatial Bioactivity of MAP Scaffolds Instructs Vasculogenesis In Vitro and Modifies Vessel Formation In Vivo, *Adv. Funct. Mater.*, 2025, **35**(3), 2400567.
83 Q. Yang, Y. Miao, J. Luo, C. Li, X. Li, Y. Chen and Y. Wang, Nanofibril-Structured Granular Hydrogels Harness Stem Cell Retention and Immunoregulation in Diabetic Microenvironment, *ACS Nano*, 2025, **19**(7), 6795–6814.
84 V. Pal, D. Gupta, S. Liu, I. Namli, S. H. A. Rizvi, Y. O. Yilmaz, L. Haugh, E. M. Gerhard and I. T. Ozbolat, Interparticle Crosslinked Ion-responsive Microgels for 3D and 4D (Bio) printing Applications, *bioRxiv*, 2025, preprint, DOI:DOI: **10.1101/2025.01.28.635095**.
85 H.-P. Lee, R. Davis Jr, T.-C. Wang, K. A. Deo, K. X. Cai, D. L. Alge, T. P. Lele and A. K. Gaharwar, Dynamically cross-linked granular hydrogels for 3d printing and therapeutic delivery, *ACS Appl. Bio Mater.*, 2023, **6**(9), 3683–3695.
86 M. Asadikorayem, F. Surman, P. Weber, D. Weber and M. Zenobi-Wong, Zwitterionic granular hydrogel for cartilage tissue engineering, *Adv. Healthcare Mater.*, 2024, **13**(25), 2301831.

87 L. Zhang, Z. Cao, T. Bai, L. Carr, J.-R. Ella-Menye, C. Irvin, B. D. Ratner and S. Jiang, Zwitterionic hydrogels implanted in mice resist the foreign-body reaction, *Nat. Biotechnol.*, 2013, **31**(6), 553–556.
88 S. Jiang and Z. Cao, Ultralow–fouling, functionalizable, and hydrolyzable zwitterionic materials and their derivatives for biological applications, *Adv. Mater.*, 2010, **22**(9), 920–932.
89 A. Ding, S. J. Lee, R. Tang, K. L. Gasvoda, F. He and E. Alsberg, 4D cell–condensate bioprinting, *Small*, 2022, **18**(36), 2202196.
90 A. Ding, O. Jeon, D. Cleveland, K. L. Gasvoda, D. Wells, S. J. Lee and E. Alsberg, Jammed micro–flake hydrogel for four–dimensional living cell bioprinting, *Adv. Mater.*, 2022, **34**(15), 2109394.
91 V. G. Muir, T. H. Qazi, J. Shan, J. r. Groll and J. A. Burdick, Influence of microgel fabrication technique on granular hydrogel properties, *ACS Biomater. Sci. Eng.*, 2021, **7**(9), 4269–4281.
92 L. S. Ribeiro, V. M. Gaspar, R. Sobreiro-Almeida, E. R. Camargo and J. F. Mano, Programmable granular hydrogel inks for 3D bioprinting applications, *Adv. Mater. Technol.*, 2023, **8**(16), 2300209.
93 A. Khademhosseini and R. Langer, Microengineered hydrogels for tissue engineering, *Biomaterials*, 2007, **28**(34), 5087–5092.
94 T. Farjami and A. Madadlou, Fabrication methods of biopolymeric microgels and microgel-based hydrogels, *Food Hydrocolloids*, 2017, **62**, 262–272.
95 C. Franco, J. Price and J. West, Development and optimization of a dual-photoinitiator, emulsion-based technique for rapid generation of cell-laden hydrogel microspheres, *Acta Biomater.*, 2011, **7**(9), 3267–3276.
96 Y. Zhu, Y. Sun, B. Rui, J. Lin, J. Shen, H. Xiao, X. Liu, Y. Chai, J. Xu and Y. Yang, A photoannealed granular hydrogel facilitating hyaline cartilage regeneration via improving chondrogenic phenotype, *ACS Appl. Mater. Interfaces*, 2022, **14**(36), 40674–40687.
97 A. E. Widener, S. Duraivel, T. E. Angelini and E. A. Phelps, Injectable microporous annealed particle hydrogel based on guest–host–interlinked polyethylene glycol maleimide microgels, *Adv. NanoBiomed Res.*, 2022, **2**(10), 2200030.
98 Q. Guan, S. Hou, K. Wang, L. Li, Y. Cheng, M. Zheng, C. Liu, X. Zhao, J. Zhou and P. Li, Micropore structure engineering of injectable granular hydrogels via controlled liquid-liquid phase separation facilitates regenerative wound healing in mice and pigs, *Biomaterials*, 2025, 123192.
99 N. D. Caprio, M. D. Davidson, A. C. Daly and J. A. Burdick, Injectable MSC spheroid and microgel granular composites for engineering tissue, *Adv. Mater.*, 2024, **36**(14), 2312226.
100 K. Nakamura, N. Di Caprio and J. A. Burdick, Engineered Shape–Morphing Transitions in Hydrogels Through Suspension Bath Printing of Temperature–Responsive Granular Hydrogel Inks, *Adv. Mater.*, 2024, **36**(47), 2410661.
101 O. Chaudhuri, L. Gu, D. Klumpers, M. Darnell, S. A. Bencherif, J. C. Weaver, N. Huebsch, H.-p. Lee, E. Lippens and G. N. Duda, Hydrogels with tunable stress relaxation regulate stem cell fate and activity, *Nat. Mater.*, 2016, **15**(3), 326–334.
102 H. Sánchez-Morán, A. Ahmadi, B. Vogler and K.-H. Roh, Oxime cross-linked alginate hydrogels with tunable stress relaxation, *Biomacromolecules*, 2019, **20**(12), 4419–4429.
103 N. Weigel, Y. Li, J. Thiele and A. Fery, From microfluidics to hierarchical hydrogel materials, *Curr. Opin. Colloid Interface Sci.*, 2023, **64**, 101673.
104 R. Riahi, A. Tamayol, S. A. M. Shaegh, A. M. Ghaemmaghami, M. R. Dokmeci and A. Khademhosseini, Microfluidics for advanced drug delivery systems, *Curr. Opin. Chem. Eng.*, 2015, **7**, 101–112.
105 B. G. Chung, K.-H. Lee, A. Khademhosseini and S.-H. Lee, Microfluidic fabrication of microengineered hydrogels and their application in tissue engineering, *Lab Chip*, 2012, **12**(1), 45–59.
106 Y. Kittel, L. P. Guerzoni, C. Itzin, D. Rommel, M. Mork, C. Bastard, B. Häßel, A. Omidinia-Anarkoli, S. P. Centeno and T. Haraszti, Varying the Stiffness and Diffusivity of Rod–Shaped Microgels Independently through Their Molecular Building Blocks, *Angew. Chem., Int. Ed.*, 2023, **62**(44), e202309779.
107 Y. Tian and E. A. Lipke, Microfluidic production of cell-laden microspheroidal hydrogels with different geometric shapes, *ACS Biomater. Sci. Eng.*, 2020, **6**(11), 6435–6444.
108 D. Rommel, M. Mork, S. Vedaraman, C. Bastard, L. P. Guerzoni, Y. Kittel, R. Vinokur, N. Born, T. Haraszti and L. De Laporte, Functionalized microgel rods interlinked into soft macroporous structures for 3D cell culture, *Adv. Sci.*, 2022, **9**(10), 2103554.
109 S. Xu, Z. Nie, M. Seo, P. Lewis, E. Kumacheva, H. A. Stone, P. Garstecki, D. B. Weibel, I. Gitlin and G. M. Whitesides, Generation of monodisperse particles by using microfluidics: control over size, shape, and composition, *Angew. Chem.*, 2005, **117**(5), 734–738.
110 J. M. de Rutte, J. Koh and D. Di Carlo, Scalable high–throughput production of modular microgels for in situ assembly of microporous tissue scaffolds, *Adv. Funct. Mater.*, 2019, **29**(25), 1900071.
111 D. M. Headen, J. R. García and A. J. García, Parallel droplet microfluidics for high throughput cell encapsulation and synthetic microgel generation, *Microsyst. Nanoeng.*, 2018, **4**(1), 1–9.
112 Y. Li, Y. Xu, Y. Liu, Z. Wang, W. Chen, L. Duan and D. Gu, Decellularized cartilage matrix scaffolds with laser-machined micropores for cartilage regeneration and articular cartilage repair, *Mater. Sci. Eng., C*, 2019, **105**, 110139.
113 T. H. Qazi, J. Wu, V. G. Muir, S. Weintraub, S. E. Gullbrand, D. Lee, D. Issadore and J. A. Burdick, Anisotropic rod–shaped particles influence injectable granular hydrogel properties and cell invasion, *Adv. Mater.*, 2022, **34**(12), 2109194.

114 C. Wang, S. Gong, H. Liu, L. Cui, Y. Ye, D. Liu, T. Liu, S. Xie and S. Li, Angiogenesis unveiled: Insights into its role and mechanisms in cartilage injury, *Exp. Gerontol.*, 2024, **195**, 112537.
115 J. M. Lowen, G. C. Bond, K. H. Griffin, N. K. Shimamoto, V. L. Thai and J. K. Leach, Multisized photoannealable microgels regulate cell spreading, aggregation, and macrophage phenotype through microporous void space, *Adv. Healthcare Mater.*, 2023, **12**(13), 2202239.
116 A. Jaberi, A. Kedzierski, S. Kheirabadi, Y. Tagay, Z. Ataie, S. Zavari, M. Naghashnejad, O. Waldron, D. Adhikari and G. Lester, Engineering Microgel Packing to Tailor the Physical and Biological Properties of Gelatin Methacryloyl Granular Hydrogel Scaffolds, *Adv. Healthcare Mater.*, 2024, **13**(25), 2402489.
117 P. P. Stankey, K. T. Kroll, A. J. Ainscough, D. S. Reynolds, A. Elamine, B. T. Fichtenkort, S. G. Uzel and J. A. Lewis, Embedding biomimetic vascular networks via coaxial sacrificial writing into functional tissue, *Adv. Mater.*, 2024, **36**(36), 2401528.
118 M. Schot, M. Becker, C. A. Paggi, F. Gomes, T. Koch, T. Gensheimer, C. Johnbosco, L. P. Nogueira, A. van der Meer and A. Carlson, Photoannealing of Microtissues Creates High–Density Capillary Network Containing Living Matter in a Volumetric–Independent Manner, *Adv. Mater.*, 2024, **36**(28), 2308949.
119 I. Cruz–Maya, R. Altobelli, M. Marrese and V. Guarino, Design of alginate based micro–gels via electro fluid dynamics to construct microphysiological cell culture systems, *Polym. Adv. Technol.*, 2021, **32**(8), 2981–2989.
120 O. Jeon, D. W. Wolfson and E. Alsberg, In situ forming growth factor-loaded coacervate microparticle-embedded hydrogel for directing encapsulated stem cell fate, *Adv. Mater.*, 2015, **27**(13), 2216.
121 A. Leferink, D. Schipper, E. Arts, E. Vrij, N. Rivron, M. Karperien, K. Mittmann, C. Van Blitterswijk, L. Moroni and R. Truckenmüller, Engineered micro-objects as scaffolding elements in cellular building blocks for bottom-up tissue engineering approaches, *Adv. Mater.*, 2014, **26**(16), 2592–2599.
122 X. Liu, J. Tao, J. Liu, X. Xu, J. Zhang, Y. Huang, Y. Chen, J. Zhang, D. Y. Deng and M. Gou, 3D printing enabled customization of functional microgels, *ACS Appl. Mater. Interfaces*, 2019, **11**(13), 12209–12215.
123 D. E. Stornello, J. Kim, Z. Chen, K. Heaton and T. H. Qazi, Controlling Microparticle Aspect Ratio via Photolithography for Injectable Granular Hydrogel Formation and Cell Delivery, *ACS Biomater. Sci. Eng.*, 2025, **10**(11), 1242–1252.
124 Y. Qian, J. Gong, K. Lu, Y. Hong, Z. Zhu, J. Zhang, Y. Zou, F. Zhou, C. Zhang and S. Zhou, DLP printed hDPSC-loaded GelMA microsphere regenerates dental pulp and repairs spinal cord, *Biomaterials*, 2023, **299**, 122137.
125 C. M. Franca, A. Athirasala, R. Subbiah, A. Tahayeri, P. Selvakumar, A. Mansoorifar, S. Horsophonphong, A. Sercia, L. Nih and L. E. Bertassoni, High–Throughput Bioprinting of Geometrically–Controlled Pre–Vascularized Injectable Microgels for Accelerated Tissue Regeneration, *Adv. Healthcare Mater.*, 2023, **12**(22), 2202840.
126 W. Yang, S. Cai, Y. Chen, W. Liang, Y. Lai, H. Yu, Y. Wang and L. Liu, Modular and customized fabrication of 3D functional microgels for bottom–up tissue engineering and drug screening, *Adv. Mater. Technol.*, 2020, **5**(5), 1900847.
127 L. H. Han, S. Yu, T. Wang, A. W. Behn and F. Yang, Microribbon–like elastomers for fabricating macroporous and highly flexible scaffolds that support cell proliferation in 3D, *Adv. Funct. Mater.*, 2013, **23**(3), 346–358.
128 Z. Ma, W. Song, D. He, X. Zhang, Y. He and H. Li, Smart μ–fiber hydrogels with macro–porous structure for sequentially promoting multiple phases of articular cartilage regeneration, *Adv. Funct. Mater.*, 2022, **32**(22), 2113380.
129 Y. Cheng, Y. Yu, F. Fu, J. Wang, L. Shang, Z. Gu and Y. Zhao, Controlled fabrication of bioactive microfibers for creating tissue constructs using microfluidic techniques, *ACS Appl. Mater. Interfaces*, 2016, **8**(2), 1080–1086.
130 B. Kessel, M. Lee, A. Bonato, Y. Tinguely, E. Tosoratti and M. Zenobi-Wong, 3D bioprinting of macroporous materials based on entangled hydrogel microstrands, *Adv. Sci.*, 2020, **7**(18), 2001419.
131 T. J. Tigner, G. Dampf, A. Tucker, Y. C. Huang, V. Jagrit, A. J. Clevenger, A. Mohapatra, S. A. Raghavan, J. N. Dulin and D. L. Alge, Clickable Granular Hydrogel Scaffolds for Delivery of Neural Progenitor Cells to Sites of Spinal Cord Injury, *Adv. Healthcare Mater.*, 2024, **13**(25), 2303912.
132 Y. Li, W. Song, L. Kong, Y. He and H. Li, Injectable and Microporous Microgel–Fiber Granular Hydrogel Loaded with Bioglass and siRNA for Promoting Diabetic Wound Healing, *Small*, 2024, **20**(19), 2309599.
133 A. M. D'Elia, O. L. Jones, G. Canziani, B. Sarkar, I. Chaiken and C. B. Rodell, Injectable Granular Hydrogels Enable Avidity-Controlled Biotherapeutic Delivery, *ACS Biomater. Sci. Eng.*, 2024, **10**(3), 1577–1588.
134 N. Hen, E. Josef, M. Davidovich-Pinhas, S. Levenberg and H. Bianco-Peled, On the Relation between the Viscoelastic Properties of Granular Hydrogels and Their Performance as Support Materials in Embedded Bioprinting, *ACS Biomater. Sci. Eng.*, 2024, **10**(10), 6734–6750.
135 A. C. Daly, Granular hydrogels in biofabrication: recent advances and future perspectives, *Adv. Healthcare Mater.*, 2024, **13**(25), 2301388.
136 V. G. Muir, S. Weintraub, A. P. Dhand, H. Fallahi, L. Han and J. A. Burdick, Influence of microgel and interstitial matrix compositions on granular hydrogel composite properties, *Adv. Sci.*, 2023, **10**(10), 2206117.
137 A. J. Seymour, D. Kilian, R. S. Navarro, S. M. Hull and S. C. Heilshorn, 3D printing microporous scaffolds from modular bioinks containing sacrificial, cell-encapsulating microgels, *Biomater. Sci.*, 2023, **11**(23), 7598–7615.
138 B. B. Mendes, A. C. Daly, R. L. Reis, R. M. Domingues, M. E. Gomes and J. A. Burdick, Injectable hyaluronic acid and platelet lysate-derived granular hydrogels for biomedical applications, *Acta Biomater.*, 2021, **119**, 101–113.

139 M. Mehrali, A. Thakur, C. P. Pennisi, S. Talebian, A. Arpanaei, M. Nikkhah and A. Dolatshahi-Pirouz, Nanoreinforced hydrogels for tissue engineering: biomaterials that are compatible with load–bearing and electroactive tissues, *Adv. Mater.*, 2017, **29**(8), 1603612.
140 Z. Liu, X. Wan, Z. L. Wang and L. Li, Electroactive biomaterials and systems for cell fate determination and tissue regeneration: design and applications, *Adv. Mater.*, 2021, **33**(32), 2007429.
141 C. Ning, Z. Zhou, G. Tan, Y. Zhu and C. Mao, Electroactive polymers for tissue regeneration: Developments and perspectives, *Prog. Polym. Sci.*, 2018, **81**, 144–162.
142 L. Chen, J. Yang, Z. Cai, Y. Huang, P. Xiao, J. Wang, F. Wang, W. Huang, W. Cui and N. Hu, Electroactive biomaterials regulate the electrophysiological microenvironment to promote bone and cartilage tissue regeneration, *Adv. Funct. Mater.*, 2024, 2314079.
143 M. Shin, K. H. Song, J. C. Burrell, D. K. Cullen and J. A. Burdick, Injectable and conductive granular hydrogels for 3D printing and electroactive tissue support, *Adv. Sci.*, 2019, **6**(20), 1901229.
144 M. Hirsch, A. Charlet and E. Amstad, 3D printing of strong and tough double network granular hydrogels, *Adv. Funct. Mater.*, 2021, **31**(5), 2005929.
145 F. Bono, S. H. Strässle Zuniga and E. Amstad, 3D Printable κ–Carrageenan–Based Granular Hydrogels, *Adv. Funct. Mater.*, 2025, **35**(3), 2413368.
146 Q. Zhang, H. Lu, N. Kawazoe and G. Chen, Pore size effect of collagen scaffolds on cartilage regeneration, *Acta Biomater.*, 2014, **10**(5), 2005–2013.
147 L. Jia, Y. Hua, J. Zeng, W. Liu, D. Wang, G. Zhou, X. Liu and H. Jiang, Bioprinting and regeneration of auricular cartilage using a bioactive bioink based on microporous photocrosslinkable acellular cartilage matrix, *Bioact. Mater.*, 2022, **16**, 66–81.
148 N. L. Claxton, M. A. Luse, B. E. Isakson and C. B. Highley, Engineering Granular Hydrogels without Interparticle Cross-Linking to Support Multicellular Organization, *ACS Biomater. Sci. Eng.*, 2024, **10**(12), 7594–7605.
149 Y. Liu, A. Suarez-Arnedo, L. Riley, T. Miley, J. Xia and T. Segura, Spatial confinement modulates macrophage response in microporous annealed particle (MAP) scaffolds, *Adv. Healthcare Mater.*, 2023, **12**(26), 2300823.
150 A. E. Widener, A. Roberts and E. A. Phelps, Granular Hydrogels for Harnessing the Immune Response, *Adv. Healthcare Mater.*, 2024, **13**(25), 2303005.
151 D. B. Emiroglu, A. Bekcic, D. Dranseike, X. Zhang, T. Zambelli, A. J. deMello and M. W. Tibbitt, Building block properties govern granular hydrogel mechanics through contact deformations, *Sci. Adv.*, 2022, **8**(50), eadd8570.
152 Z. Ataie, S. Kheirabadi, J. W. Zhang, A. Kedzierski, C. Petrosky, R. Jiang, C. Vollberg and A. Sheikhi, Nanoengineered granular hydrogel bioinks with preserved interconnected microporosity for extrusion bioprinting, *Small*, 2022, **18**(37), 2202390.
153 L. Pruett, R. Ellis, M. McDermott, C. Roosa and D. Griffin, Spatially heterogeneous epidermal growth factor release from microporous annealed particle (MAP) hydrogel for improved wound closure, *J. Mater. Chem. B*, 2021, **9**(35), 7132–7139.
154 K. Wang, Z. Wang, H. Hu and C. Gao, Supramolecular microgels/microgel scaffolds for tissue repair and regeneration, *Supramol. Mater.*, 2022, **1**, 100006.
155 J. Zheng, C. Zhu, X. Xu, X. Wang and J. Fu, Supramolecular assemblies of multifunctional microgels for biomedical applications, *J. Mater. Chem. B*, 2023, **11**(27), 6265–6289.
156 Y. L. Han, Y. Yang, S. Liu, J. Wu, Y. Chen, T. J. Lu and F. Xu, Directed self-assembly of microscale hydrogels by electrostatic interaction, *Biofabrication*, 2013, **5**(3), 035004.
157 R. S. Hsu, P. Y. Chen, J. H. Fang, Y. Y. Chen, C. W. Chang, Y. J. Lu and S. H. Hu, Adaptable microporous hydrogels of propagating NGF–gradient by injectable building blocks for accelerated axonal outgrowth, *Adv. Sci.*, 2019, **6**(16), 1900520.
158 J. Yang, Y. S. Zhang, K. Yue and A. Khademhosseini, Cell-laden hydrogels for osteochondral and cartilage tissue engineering, *Acta Biomater.*, 2017, **57**, 1–25.
159 A. S. Caldwell, G. T. Campbell, K. M. Shekiro and K. S. Anseth, Clickable microgel scaffolds as platforms for 3D cell encapsulation, *Adv. Healthcare Mater.*, 2017, **6**(15), 1700254.
160 J. Zhang, Y. Zeng, Y. Heng, Y. Shen, X. Sun, Y. Wang, H. Zheng, M. Zeng and Z. Yu, Enzyme–assisted activation technique for producing versatile hydrogel microparticle scaffolds with high surface chemical reactivity, *Adv. Funct. Mater.*, 2024, **34**(30), 2400858.
161 D. R. Griffin, W. M. Weaver, P. O. Scumpia, D. Di Carlo and T. Segura, Accelerated wound healing by injectable microporous gel scaffolds assembled from annealed building blocks, *Nat. Mater.*, 2015, **14**(7), 737–744.
162 O. Jeon, Y. B. Lee, T. J. Hinton, A. W. Feinberg and E. Alsberg, Cryopreserved cell-laden alginate microgel bioink for 3D bioprinting of living tissues, *Mater. Today Chem.*, 2019, **12**, 61–70.
163 A. S. Caldwell, B. A. Aguado and K. S. Anseth, Designing microgels for cell culture and controlled assembly of tissue microenvironments, *Adv. Funct. Mater.*, 2020, **30**(37), 1907670.
164 A. J. Seymour, S. Shin and S. C. Heilshorn, 3D printing of microgel scaffolds with tunable void fraction to promote cell infiltration, *Adv. Healthcare Mater.*, 2021, **10**(18), 2100644.
165 B. Conrad, L.-H. Han and F. Yang, Gelatin-based microribbon hydrogels accelerate cartilage formation by mesenchymal stem cells in three dimensions, *Tissue Eng., Part A*, 2018, **24**(21–22), 1631–1640.
166 C. Gegg and F. Yang, Spatially patterned microribbon-based hydrogels induce zonally-organized cartilage regeneration by stem cells in 3D, *Acta Biomater.*, 2020, **101**, 196–205.

167 F. Li, V. X. Truong, H. Thissen, J. E. Frith and J. S. Forsythe, Microfluidic encapsulation of human mesenchymal stem cells for articular cartilage tissue regeneration, *ACS Appl. Mater. Interfaces*, 2017, **9**(10), 8589–8601.

168 S. Sarvepalli, S. R. Pasika, S. Vadarevu, S. Bolla and P. K. Bolla, A comprehensive review on injectable hydrogels for cell therapy, *J. Drug Delivery Sci. Technol.*, 2025, 106648.

169 Q. Feng, Q. Li, H. Wen, J. Chen, M. Liang, H. Huang, D. Lan, H. Dong and X. Cao, Injection and self–assembly of bioinspired stem cell–laden gelatin/hyaluronic acid hybrid microgels promote cartilage repair in vivo, *Adv. Funct. Mater.*, 2019, **29**(50), 1906690.

170 Y. Ma, H. Yang, X. Zong, J. Wu, X. Ji, W. Liu, P. Yuan, X. Chen, C. Yang and X. Li, Artificial M2 macrophages for disease-modifying osteoarthritis therapeutics, *Biomaterials*, 2021, **274**, 120865.

171 M. Asadikorayem, L. G. Brunel, P. Weber, S. C. Heilshorn and M. Zenobi-Wong, Porosity dominates over microgel stiffness for promoting chondrogenesis in zwitterionic granular hydrogels, *Biomater. Sci.*, 2024, **12**(21), 5504–5520.

172 Z. Lin, Q. Li, X. Han, H. Luo, Z. Wang, Z. Qin, Y. Huang, Q. Feng and X. Cao, An injectable and degradable heterogeneous microgel assembly capable of forming a "micronest group" for cell condensation and cartilage regeneration, *Mater. Horiz.*, 2024, **11**(21), 5438–5450.

173 S. Brown, S. Kumar and B. Sharma, Intra-articular targeting of nanomaterials for the treatment of osteoarthritis, *Acta Biomater.*, 2019, **93**, 239–257.

174 J. Pradal, P. Maudens, C. Gabay, C. A. Seemayer, O. Jordan and E. Allémann, Effect of particle size on the biodistribution of nano-and microparticles following intra-articular injection in mice, *Int. J. Pharm.*, 2016, **498**(1–2), 119–129.

175 Q. Feng, D. Li, Q. Li, S. Li, H. Huang, H. Li, H. Dong and X. Cao, Dynamic nanocomposite microgel assembly with microporosity, injectability, tissue–adhesion, and sustained drug release promotes articular cartilage repair and regeneration, *Adv. Healthcare Mater.*, 2022, **11**(8), 2102395.

176 A. Puiggalí-Jou, M. Asadikorayem, K. Maniura-Weber and M. Zenobi-Wong, Growth factor–loaded sulfated microislands in granular hydrogels promote hMSCs migration and chondrogenic differentiation, *Acta Biomater.*, 2023, **166**, 69–84.

177 V. Muir, M. Fainor, B. Orozco, R. Hilliard, M. Boyes, H. Smith R. Mauck, T. Schaer, J. Burdick and S. Gullbrand, Injectable Radiopaque Hyaluronic Acid Granular Hydrogels for Intervertebral Disc Repair, *Adv. Healthcare Mater.*, 2023, **13**, 2303326.

178 M. Said, C. Tavakoli, C. Dumot, K. Toupet, C. Olivier, A. Gilles, M. Maumus, Y. C. Dong, N. Collomb and C. Auxenfans, A self-healing radiopaque hyaluronic acid hydrogel as a new injectable biomaterial for precision medicine in osteoarthritis, *Theranostics*, 2025, **15**(9), 4054.

179 O. Jeon, H. Park, J. K. Leach and E. Alsberg, Biofabrication of engineered tissues by 3D bioprinting of tissue specific high cell-density bioinks, *Mater. Today*, 2025, **86**, 172–182.

180 P. Luo, R. Huang, Y. Wu, X. Liu, Z. Shan, L. Gong, S. Deng, H. Liu, J. Fang and S. Wu, Tailoring the multiscale mechanics of tunable decellularized extracellular matrix (dECM) for wound healing through immunomodulation, *Bioact. Mater.*, 2023, **28**, 95–111.

181 K. Zhang, Y. Zhu, T. Wang, D. Niu, Z. Xiahou, H. Wang, P. Fu, H. Cui and J. Yin, Lubricating Micro–Interface Assisted General Strategy for Preparing dECM–Microparticle–Based Heterogeneous Granular Inks toward 3D Printing, *Adv. Funct. Mater.*, 2024, **34**(11), 2307886.

182 K. Flégeau, A. Puiggali-Jou and M. Zenobi-Wong, Cartilage tissue engineering by extrusion bioprinting utilizing porous hyaluronic acid microgel bioinks, *Biofabrication*, 2022, **14**(3), 034105.

183 F. Surman, M. Asadikorayem, P. Weber, D. Weber and M. Zenobi-Wong, Ionically annealed zwitterionic microgels for bioprinting of cartilaginous constructs, *Biofabrication*, 2024, **16**(2), 025004.

184 F. Momeni, M. Mehdi, N.,S. Hassani, X. Liu and J. Ni, A review of 4D printing, *Mater. Des.*, 2017, **122**, 42–79.

185 N. Ashammakhi, S. Ahadian, F. Zengjie, K. Suthiwanich, F. Lorestani, G. Orive, S. Ostrovidov and A. Khademhosseini, Advances and future perspectives in 4D bioprinting, *Biotechnol. J.*, 2018, **13**(12), 1800148.

186 G. D. DuRaine, W. E. Brown, J. C. Hu and K. A. Athanasiou, Emergence of scaffold-free approaches for tissue engineering musculoskeletal cartilages, *Ann. Biomed. Eng.*, 2015, **43**, 543–554.

187 A. D. Dikina, D. S. Alt, S. Herberg, A. McMillan, H. A. Strobel, Z. Zheng, M. Cao, B. P. Lai, O. Jeon and V. I. Petsinger, A modular strategy to engineer complex tissues and organs, *Adv. Sci.*, 2018, **5**(5), 1700402.

188 N. Di Caprio, A. J. Hughes and J. A. Burdick, Programmed shape transformations in cell-laden granular composites, *Sci. Adv.*, 2025, **11**(3), eadq5011.

189 A. Mandal, J. Clegg, A. Anselmo and S. Mitragotri, Hydrogels in the clinic, *Bioeng. Transl. Med.*, 2020, **5**(2), e10158.

190 Y. Kittel, A. J. Kuehne and L. De Laporte, Translating therapeutic microgels into clinical applications, *Adv. Healthcare Mater.*, 2022, **11**(6), 2101989.

191 C. V. Oberweis, J. A. Marchal, E. López-Ruiz and P. Gálvez-Martín, A worldwide overview of regulatory frameworks for tissue-based products, *Tissue Eng., Part B*, 2020, **26**(2), 181–196.

192 W. J. Seeto, Y. Tian, S. Pradhan, P. Kerscher and E. A. Lipke, Rapid production of cell–laden microspheres using a flexible microfluidic encapsulation platform, *Small*, 2019, **15**(47), 1902058.