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Review article

Microgel-based bioink for extrusion-based 3D bioprinting and its applications in tissue engineering

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ABSTRACT

Extrusion-based 3D bioprinting is being increasingly adopted as a versatile biofabrication method for making biomimetic constructs in tissue engineering. However, the lack of ideal bioinks continues to limit its broader application. Conventional hydrogel-based bioinks typically possess a densely crosslinked nanoporous structure that hinders their ability to fully support cell behavior. Microgel-based bioinks have recently emerged as a promising alternative due to their enhanced printability and functionality. This review will begin with the evolution of the "bioink" concept, followed by a discussion on bioink categories and the requirements of ideal bioinks. It will then introduce hydrogel-based bioinks and their limitations, followed by a definition of microgels and microgel-based bioinks and a discussion of their key properties, highlighting their differences compared to conventional hydrogel-based bioinks. Topics on microgel-based bioinks are then presented in order of the printing process: pre-printing (fabrication of microgels and formulation of microgel-based bioinks), during printing and post-printing (microgel assembly kinetics). Uniquely, this review will examine the various applications of microgel-based bioinks in tissue engineering, summarizing their advantages and limitations. Finally, the current challenges and future perspectives of using microgel-based bioinks are discussed. This review comprehensively examines microgel-based bioinks for 3D bioprinting, highlighting their potential to overcome current challenges and setting the stage for their future applications in creating complex, functional tissue engineering scaffolds.

1. Introduction

Over the past decade, 3D bioprinting has emerged as a pivotal technology in biomedical engineering, with bioinks serving as the core material [1–5]. 3D bioprinting is an advanced biofabrication technology that creates complex, biologically functional, biomimetic constructs using various techniques, including layer-by-layer deposition, volumetric bioprinting, and other approaches [6–9]. This technology offers the ability to mimic natural tissue systems by controlling the spatial arrangement of cells, allowing for the creation of heterogeneous constructs using different cells and bioinks [10]. Continuous advances in 3D bioprinting technology have significant economic relevance. As indicated by a report from Grand View Research, the 3D bioprinting market was valued at USD 1.4 billion in 2020, with a projected compound annual growth rate of 15.8 % from 2021 to 2028. In 2024, the 3D bioprinting market is estimated to occupy approximately 10 % of the overall 3D printing market [11]. Despite notable progress in various bioprinting modalities [12], their practical applications and particularly the translatability of bioprinted products have been limited by the lack of suitable bioinks. These limitations arise from the persistent challenge

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of balancing the requirements between printability and functionality of bioink. Printability refers to the bioink's ability to be successfully printed, while functionality encompasses various attributes and performance metrics of the bioink, including biocompatibility, microporosity, shape fidelity, tunability, heterogeneity, and other key features [13].

Hydrogels, composed of 3D hydrophilic polymer networks, are frequently chosen for bioink formulation due to their printability. However, conventional hydrogel-based bioinks are severely limited by their dense nanoporous networks that restrict the transport of key biomolecules and impair normal cellular [14–17]. Microgels are modular microscale hydrogel units, comprising polymer chains crosslinked within small dimensions. They have been widely used in various biomedical applications over the last decade [18–21]. Recently, microgels have been utilized as building blocks for creating microporous hydrogels through microgel assembly methods, addressing the limitations of the nanoporous structures in conventional hydrogel scaffolds [22,23]. When assembled, the voids between packed microgels form a microporous structure that promotes natural cell behavior. Besides their microporosity, microgels are found to possess improved biocompatibility, tunability, and the ability to precisely adjust their physical, chemical, and mechanical properties to meet specific requirements [24,25]. This makes them ideal alternatives to existing hydrogels for formulating bioinks. However, the exploration of microgels as bioinks for 3D bioprinting is still in its early stages.

Numerous studies have investigated the role of microgels in the biomedical sciences, leading to the publication of several insightful review articles. These reviews predominantly focus on the materials used to synthesize microgels, the techniques for their production, methods for assembling microgels into microporous scaffolds, and their applications in biomedical engineering, such as cell delivery, drug delivery, and tissue engineering scaffolds [24,26–29]. Recently, the potential of microgels as bioinks for 3D bioprinting has garnered growing interest. A few reviews have begun to address this topic, primarily emphasizing the fabrication of microgels and the formulation of microgel-based bioinks [30,31]. For instance, An et al. [30] reviewed microparticulate inks for bioprinting, covering polymer microparticulate inks, tissue-derived microparticles, and bioactive inorganic microparticles. However, their discussion of microgel-based bioinks represents only a small portion of the content. Similarly, Ribeiro et al. [31] highlighted the emergence of granular inks comprising jammed hydrogel building blocks (microgel-based bioinks) as programmable precursors for 3D bioprinting. Their review delves into the fabrication techniques for microgels, the jamming process, and how these techniques influence the ink's final properties, concluding with a focus on cell–granular hydrogel interactions in biomedical applications. Despite these contributions, existing reviews often fall short in two key areas. First, they lack a detailed comparative analysis of microgel-based bioinks versus conventional hydrogel-based bioinks, particularly in addressing the limitations of the latter. Second, they fail to comprehensively explore the applications of microgel-based bioinks across diverse domains of tissue engineering. This gap in the literature underscores the need for a new review that provides a deeper and more balanced examination of microgel-based bioinks, their advantages, and their transformative potential in 3D bioprinting.

This review highlights the significant potential of microgel-based bioinks in 3D bioprinting and their applications in tissue regeneration. It begins by outlining the evolution of the "bioink" concept, followed by a discussion on bioink categories and the requirements of ideal bioinks. The review then examines traditional hydrogel-based bioinks and their limitations, defining key properties of microgels and microgel-based bioinks. We emphasize the advantages of microgel-based bioinks by comparing their principles, printability, and biological functionality with traditional hydrogel-based bioinks and provide examples of their current applications in printing tissue engineering scaffolds using extrusion-based bioprinting technology. This review systematically explores the synthesis, properties, and applications of microgel-based bioinks throughout the bioprinting stages (pre-printing, during-printing, and post-printing), offering unique insights into a rapidly evolving field to inspire further innovation in bioink formulations for biomedical applications.

2. Bioink

2.1. The concept of "bioink"

3D bioprinting involves a broad range of bioprinting techniques and bioinks. The definition of bioink has evolved significantly with advances over time in bioprinting technology and its applications [32]. In 2003, Mironov et al. were the first to propose the term "bioink" for describing cells and "biopaper" for describing hydrogels during the process of printing living tissues [33]. They initially printed the "biopaper" (hydrogel) by 3D printing, and then seeded the "bioink" (cells) onto or within the "biopaper" by bioprinting. In this study, the term "bioink" originally referred to the cellular component that was positioned on or within hydrogels, while the hydrogels and cells were separated during the bioprinting process. After this study, cells and cell aggregates have been widely used as bioinks in several pioneering studies [33–35].

With further advances in bioprinting technology, particularly with the growing use of direct-write extrusion-based printing, the knowledge base has been expanded in understanding the rheological properties of materials used in the printing process. This increased understanding has led to a unified concept of bioink as the dispensed material, leaving behind the concept of "biopaper" [32]. In contemporary bioprinting, the inks for biofabrication are distinguished into "bioinks" and "biomaterial inks" [36] or scaffold-free bioink and scaffold-based bioink [37], as shown in Fig. 1A. According to these definitions, a "bioink" contains cells as a mandatory component while biomaterials are an optional component. Single cells, coated cells, or cell aggregates can be used as bioinks without exogenous biomaterial, mimicking the process of embryonic development [38,39]. In this process, neo-tissues are established from cells deposited in specific patterns to fuse and mature, ultimately forming larger-scale functional tissues. Meanwhile, in a "biomaterial ink", only biomaterials are used to fabricate scaffolds through a 3D bioprinting process. After the printed scaffolds are obtained, cells can be seeded on or within the scaffolds to create engineered tissue constructs. The cell-seeded scaffolds may be subjected to *in vitro* culture for a period of time before being implanted into tissue defects, or directly implanted *in vivo* without pre-culture. Because scaffolds printed using biomaterial ink do not initially contain living cells, they have less stringent physicochemical demands and thus allow a much wider window of processing parameters. At minimum, the scaffolds must be biocompatible and have certain bioactive properties to support cell growth and tissue formation [40,41].

In the current bioink literature, there is no clear gap between these two types of inks, since a cell-based bioink usually needs some biomaterial to offer protection from shear forces and to support the shape fidelity and mechanical properties of the printed constructs, while a biomaterial-based ink may need cells to provide biological activities and facilitate tissue regeneration [43]. Thus, the definition of bioink has evolved from simple "biopaper" to sophisticated, multi-component systems (Fig. 1B) designed to create functional, living tissues [42,44]. This evolution reflects the increasing complexity and capabilities of 3D bioprinting and its expanding role in tissue engineering, regenerative medicine, and biomedical research. Today, bioinks are comprehensively defined as composite materials used in bioprinting that can be solely biomaterials, solely living cells, combination of biomaterials and living cells, combination of biomaterials and bioactive components, or combination of biomaterials, cells and bioactive components [45]. They are designed to be printable, to support cell viability and function, and to facilitate tissue formation and regeneration [36].

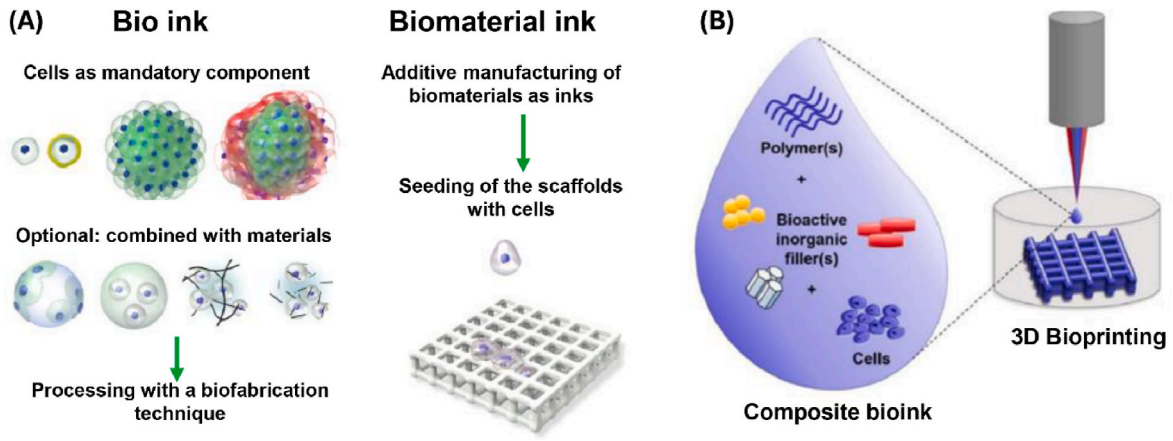


Fig. 1. Definition of bioink and its evolution. (A) The traditional concepts of bioink and biomaterial ink. Reproduced under the terms of the Creative Commons CC-BY-NC-ND license [36]. Copyright 2020, American Chemical Society. (B) The current concept of composite bioink. Reproduced under the terms of the Creative Commons CC-BY license [42]. Copyright 2022, by the Authors, MDPI, Basel, Switzerland.

2.2. Bioink categories

Bioinks are now classified according to various criteria, broadly into five categories depending on their components, functionality, and complexity: biomaterial bioinks, cell-laden bioinks, functional bioinks, advanced bioinks, and smart bioinks. ***Biomaterial bioinks,*** or cell-free bioinks, only contain biomaterial. They were basic and simple bioinks developed in the early stage of bioprinting technology for creating structures that could support cell attachment and growth post-printing [46]. The term "biomaterial bioink" now refers to the hydrogel precursors or aqueous polymer formulation that can be printed into a construct for subsequent cell seeding or implantation [47]. With advances in bioprinting technology, ***cell-laden bioinks*** emerged to include living cells suspended within the biomaterial. This development marked a significant shift in bioprinting, emphasizing the ability to directly print living tissues [48,49]. The next stage in technological evolution involved bioinks that contained not only cells but also additional bioactive components such as growth factors, signaling molecules, and extracellular matrix (ECM) components, broadly categorized into ***functional bioinks***. These bioinks aimed to more closely mimic the natural cellular environment, and actively enhance cell viability and function [50]. Recently, ***advanced bioinks*** comprising multi-material formulations have been developed, capable of printing complex tissue structures with heterogeneous or anisotropic properties. For example, modular bioinks containing small building blocks such as microgels can offer more precise control over the architecture and mechanical properties of the printed construct [44]. ***Smart bioinks*** are the latest advances equipped with the ability to undergo property changes in response to external stimuli, such as temperature, pH, and light. They enable dynamic tuning of the printed constructs post-fabrication, thereby improving functionality and integration with host tissues [42,51]. Over the evolution of bioink definition and categories, the biomaterial component remains the fundamental element of all bioinks, which will be the focus of this review.

2.3. The ideal biomaterials for a bioink

Biomaterial selection to make a bioink for 3D bioprinting is informed by the specific intended application, type(s) of cells involved, and type of bioprinter to be employed [52]. Generally, the key attributes of an ideal biomaterial for a bioink should include ***printability*** and ***functionality*** [36,53], which is summarized in Fig. 2.

Printability refers to the ability of the bioink to be extruded or deposited through a bioprinter nozzle. To meet this requirement, the biomaterial should have appropriate rheological properties including

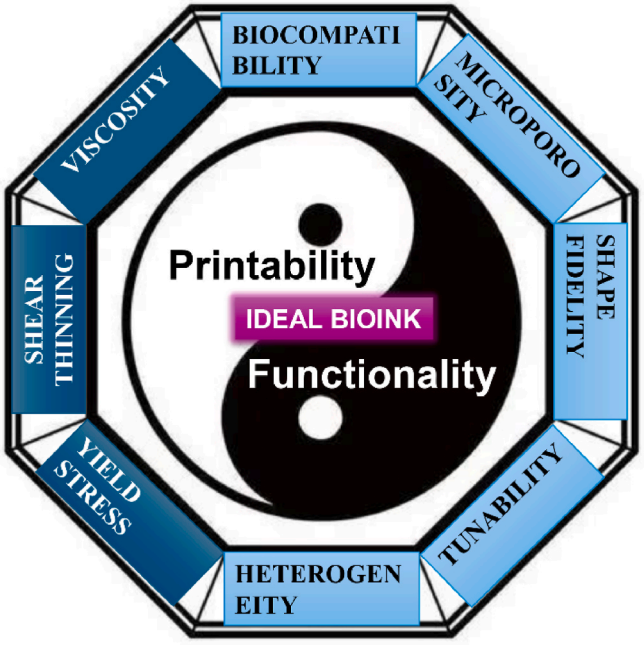


Fig. 2. Requirements of an ideal bioink for 3D bioprinting.

desired viscosity and shear thinning/shear recovery capabilities, as well as the ability to be crosslinked into stable structures in the post-printing stage. As an example, a bioink for use in extrusion-based bioprinting should allow for filament formation. This requires the biomaterial of the bioink to have non-Newtonian effects such as strong shear-thinning followed by quick structural recovery, important for reducing cell damage during printing while maintaining the ability of the material to be stacked [54]. Furthermore, the bioink material should have ***functionality*** for specific applications, including physical functionality and biological functionality. For instance, it should have appropriate stiffness and elasticity to maintain the shape fidelity of the construct immediately after printing, and provide structural support during the period of tissue regeneration [55]. Meanwhile, the bioink should be compatible with living cells, ideally providing chemical, structural or mechanical cues that promote cell survival and function, including adherence, spreading, proliferation and differentiation, and/or modulate the host inflammatory response, collectively leading to accelerated tissue formation [56]. Additionally, the material component(s) of a

bioink should not be immunogenic and have the ability to degrade at a rate matching tissue regeneration with non-toxic degradation products [57].

3. Hydrogel-based bioinks and their limitations

3.1. Traditional hydrogel-based bioinks

Hydrogels are highly attractive materials for bioink formulation. Within a hydrogel, polymers are crosslinked into a solid 3D network that can swell in water. The hydrophilic nature of the polymers allows the hydrogel to retain a large amount of liquid, contributing to distinct properties [58,59]. Hydrogels are commonly used as bioinks in 3D bioprinting due to their excellent biocompatibility, high water content, adjustable printability, tunable properties, ease of crosslinking, similarities in composition and structure to the natural ECM, ability to encapsulate and deliver bioactive molecules, and biodegradability [16,60–62].

Traditional hydrogel-based bioinks typically consist of hydrogel precursor solutions with specific viscosities. Two main types of hydrogel-based bioinks have been widely used and extensively discussed in recent reviews [13,63–65]: 1) ***Cell-free hydrogel-based bioink,*** where hydrogel precursors are printed directly into scaffolds. These scaffolds can either serve as platforms for *in vitro* cell culture or be implanted *in vivo*, with or without cells seeded post-printing, to promote tissue regeneration. 2) ***Cell-laden hydrogel-based bioink***, where hydrogel precursors are mixed with living cells for printing into cellular constructs. For both types of hydrogel-based bioinks, once printed and deposited onto a collector, the intended structure is formed through crosslinking of the hydrogel precursor [36]. Various biological components such as growth factors, DNA, miRNA, cytokines, and extracellular vesicles can be incorporated into the hydrogel precursor to enhance the bioactivity of the bioink and accelerate tissue regeneration [45].

To date, hydrogel-based bioinks are the most frequently used in 3D bioprinting, with several products being commercially available as summarized in Table 1. All existing products are cell-free bioinks, but some are enriched with growth factors, such as bone morphogenetic proteins (BMPs) in Gel4Cell-BMP to promote bone regeneration, vascular endothelial growth factor (VEGF) in Gel4Cell-VEGF to stimulate angiogenesis and transforming growth factor (TGF) in Gel4Cell-TGF to induce chondrogenesis. Additionally, all products are formulated using naturally derived polymers, including gelatin, sodium alginate (SA), collagen, and hyaluronic acid. Among the commercial suppliers in this space, Bioink Solutions Inc. Offers the most diverse range of bioink products, formulated using gelatin with or without the addition of growth factors.

Table 1
Commercially available hydrogel-based bioinks.

Product Name	Materials	Crosslink method	Biological components	Company
Gel4Cell [66]	Gelatin	Photo crosslinking	Nil	Bioink Solutions, Inc
Gel4Cell-BMP	Gelatin	Photo crosslinking	BMP	Bioink Solutions, Inc
Gel4Cell-VEGF	Gelatin	Photo crosslinking	VEGF	Bioink Solutions, Inc
Gel4Cell-TGF	Gelatin	Photo crosslinking	TGF	Bioink Solutions, Inc
CELLINK [67]	Sodium Alginate	Ionic crosslinking	Nil	CELLINK
GEL-MA INX	Gelatin	Photo crosslinking	Nil	BIO INX
HYDROBIO INX	Gelatin	Photo crosslinking	Nil	BIO INX
EASYGEL INX	Gelatin	Photo crosslinking	Nil	BIO INX
Lifeink 260	Type I collagen	Thermal crosslinking	Nil	Advanced BioMatrix
PhotoHA-INK	Methacrylated hyaluronic acid	Photo crosslinking	NA	Advanced BioMatrix

3.2. Limitations of traditional hydrogel-based bioinks

Traditional hydrogel-based bioinks have enabled rapid advances in 3D bioprinting technology over the last two decades, but the resulting scaffolds face significant challenges in being translated for clinical applications in tissue regeneration. Printability remains a primary issue of hydrogel-based bioinks, where despite shear-thinning and shear-recovery properties, they often exhibit suboptimal shape fidelity during printing. The bioink is in a liquid state before crosslinking, preventing it from adequately supporting subsequent layers. Hence, traditional hydrogel-based bioinks are generally unsuitable for printing structures exceeding a few millimeters in height as they either crosslink too slowly or lack sufficient strength. Moreover, the random crosslinking within hydrogel bioinks can lead to stress concentrations on less extensible chains during deformation [68]. Efforts have been made to reinforce hydrogel bioinks by increasing polymer content or crosslinking density, although these approaches often result in undesirable side effects such as reduced permeability and porosity, which in turn can negatively affect cell growth and tissue regeneration [69,70].

Hydrogel-based bioinks form a nanoporous structure in the printed construct following crosslinking, now being increasingly recognized as the primary factor limiting scaffold functionality in tissue engineering. During crosslinking, emerging spaces between entangled polymer chains shape the porous structure of the hydrogel. Due to the covalent bonding between polymer chains, these spaces are typically in the nanoscale range (mean pore size ≈5 nm), leading to the characteristic nanoporous structure of conventional hydrogels [71,72]. When hydrogel-based bioinks are used to encapsulate cells, crosslinking results in a nanoporous structure that physically restricts the cells, limiting normal functions such as spreading, migration, and cell-cell interactions [73]. In the case of cell-free bioinks, the nanoporous structure of printed constructs hinders the infiltration of endogenous cells and vasculature after *in vivo* implantation [74], leading to slow cell recruitment, delayed angiogenesis, and poor integration with host tissues [53,75,76]. Additionally, the nanoporous structure impedes the exchange of nutrients and metabolic waste, further affecting normal cell behavior while slowing hydrogel degradation and hence limiting the space available for cell migration and tissue infiltration [77–79].

Thus, the key challenge in hydrogel-based bioink development is to achieve an optimal balance between functionality and printability, often referred to as the “biofabrication window”. This balance reflects a compromise between biological performance (e.g., cell behavior) and printability, which are influenced by factors such as polymer concentration, viscosity, crosslink density, and temperature [80,81]. For instance, bioinks with high polymer concentration, viscosity, and crosslinking density typically exhibit high shape fidelity in printed constructs. However, these constructs often have small pore sizes and low porosity, significantly restricting cell proliferation, migration, and differentiation [58]. To address these limitations and expand the biofabrication window, new approaches such as microgel-based bioinks have recently emerged [28,82–84].

4. Microgel-based bioinks and their comparison with traditional hydrogel-based bioinks

4.1. Definition of microgels

Hydrogels were originally classified by particle size as macrogels (>100 μm), microgels (100 nm-100 μm), or nanogels (<100 μm) [85].

With advances in microgel research, the term "macrogel" has been replaced by "bulky hydrogel", and hydrogel particles ranging from 100 nm to several hundred micrometers are now collectively referred to as microgels [86]. Microgels are small hydrogel units, comprising polymer chains crosslinked within small dimensions. They are larger than individual polymer chains (nanogels) but significantly smaller than traditional bulky hydrogels, which typically range from millimeters to centimeters in size.

4.2. Category of microgel-based bioinks

A microgel-based bioink is a type of bioink used in 3D bioprinting, composed of microgels that can form either a jammed network of pure microgels or a suspension of microgels within a supporting liquid, typically a hydrogel precursor solution [87,88]. These two formulation methods lead to two different types of microgel-based bioinks, the jammed microgel-based bioink and the granular microgel-based bioink. The primary difference between jammed microgel-based bioinks and granular microgel-based bioinks lies in the physical state and behavior of the microgels within the bioink. In the jammed microgel-based bioinks, the microgels are tightly packed and in a jammed state (details in section 6), where their mechanical interactions prevent them from freely moving relative to one another. In the granular microgel-based bioinks, the microgels are in a loosely packed or granular state, with more freedom to move relative to each other [30,89–92].

These differences lead to several distinctions in the properties of these two types of microgel-based bioinks. In terms of rheological properties, jammed microgel-based bioink usually exhibits yield stress behavior, meaning it remains solid-like until sufficient stress is applied to overcome the jammed configuration. It provides excellent shape fidelity after extrusion and supports complex 3D structures. However, granular microgel-based bioinks may exhibit flowability or partial solid-like behavior, depending on the packing density of the microgels. It can be designed to behave more fluid-like for easy handling or less rigid applications. Additionally, after the bioinks are printed into constructs, the porosity of the constructs is significantly affected by the status of microgels in the bioinks. The packing density of microgels in the jammed microgel-based bioinks decreases void spaces between microgels, leading to smaller pore sizes. This can limit cell infiltration initially but supports surface interactions well. In contrast, the printed constructs with granular microgel-based bioink typically exhibits larger void spaces due to looser packing of microgels, promoting better initial cell infiltration and nutrient diffusion [91,93,94].

Although the two types of microgel-based bioinks have been tried in 3D bioprinting for different applications, jammed microgel-based bioink is preferred for applications requiring high precision and shape fidelity, such as intricate tissue scaffolds or mechanically stable constructs immediately post-printing while granular microgel-based bioink is more suitable for biological integration and dynamic environments, such as applications emphasizing cell encapsulation, migration, and nutrient diffusion [75,82,95]. In summary, jammed microgel-based bioinks prioritize printability and structural integrity due to their solid-like behavior while granular microgel-based bioinks emphasize biological functionality and versatility, leveraging their flowability and porosity for cell-focused applications.

4.3. Printability

Recent studies have highlighted the significant potential of using microgels to formulate bioinks, largely due to their unique properties including shear-thinning, shear-recovery, resilience to shear forces, and adaptability to various material formulations and crosslinking strategies [18,96,97]. While both conventional hydrogel-based bioinks and emerging microgel-based bioinks exhibit rheological properties, they do so through distinct mechanisms due to differences in structural composition [98]. This section will discuss the printability characteristics of microgel-based bioinks, focusing on factors such as shear-thinning, shear recovery, and yield stress [99], and compare with hydrogel-based bioinks by examining the mechanisms governing their printability.

4.3.1. Viscosity and shear-thinning

Viscosity is defined as the inherent resistance exhibited by a fluid to flow when subjected to external stress. Viscosity plays a central role in determining bioink performance during and after the 3D bioprinting process. It affects printability, resolution, structural integrity, cell viability, and the mechanical properties of the final construct in various stages of bioprinting [100]. The viscosity of microgel-based and hydrogel-based bioinks are governed by different mechanisms. Conventional hydrogel-based bioink has ***polymer network-based viscosity*** [101]. Since they consist of dispersed/entangled polymer chains suspended in a liquid medium, bioink viscosity is derived from the entanglement and crosslinking of polymer chains. Thus, the chemical nature of the polymer, including the presence of side groups, hydrophilicity, and molecular weight influence hydrogel-based bioink viscosity. Additionally, the viscosity of bulk hydrogel bioink is influenced by the properties of the polymer precursor solution, including the concentration and degree of polymer crosslinking [19]. In contrast, microgel bioink has ***particle-based viscosity***. As they consist of dispersed microgel particles, bioink viscosity arises primarily from the interaction and packing of these particles [102]. The concentration (volume fraction) of microgel particles and their size distribution significantly influence the viscosity. Higher concentrations of microgels with optimal particle sizes can lead to increased viscosity due to enhanced particle-particle interactions. In addition, the nature of interactions between the microgel particles, including steric hindrance, electrostatic forces, and hydrogen bonding, contribute to the overall viscosity.

Shear-thinning, commonly known as pseudo-plastic behavior, is a distinct non-Newtonian phenomenon where the viscosity of a material decreases in response to increasing shear rates [103]. Microgel-based bioinks and conventional hydrogel-based bioinks exhibit shear-thinning behavior through different mechanisms due to their distinct structural compositions and viscosity formation mechanisms [30]. Bulk hydrogel bioinks are composed of long polymer chains that form a network through entanglement and crosslinking. At low or zero shear stress, the polymer chains form a highly entangled or crosslinked network, contributing to high viscosity. Under shear stress, disruption of the polymer network allows the chains to move more freely relative to each other and align in the direction of the flow. This alignment reduces entanglements and internal friction, leading to decreased viscosity [19]. Under this mechanism, the water content within the hydrogel network affects its shear-thinning properties. Well-hydrated hydrogels with a higher water content can exhibit more pronounced shear-thinning, since the polymer chains can more easily align and move past each other under shear stress. Different from conventional hydrogel-based bioinks, microgel-based bioinks contain a dispersion of microgel particles, where the particles are often soft and deformable. At low or zero shear stress before printing, the microgel particles form a network structure through physical interactions such as van der Waals forces, hydrogen bonding, or electrostatic interactions, leading to higher viscosity. Under shear stress, disruption of these interactions allows the particles to slide past each other more easily and the microgel particles can realign and deform, resulting in reduced viscosity. Thus, the concentration of microgel particles (volume fraction) plays a crucial role in the shear-thinning behavior of microgel-based bioinks. Higher particle concentrations enhance shear-thinning behavior because the increased frequency of particle-particle interactions leads to structural rearrangements under shear. As the particles align into more streamlined configurations, the resistance to flow decreases, resulting in a more pronounced reduction in viscosity.

The intrinsic differences between microgel-based bioinks and conventional hydrogel-based bioinks lead to variations in viscosity and

shear-thinning behavior. In microgel-based bioinks, the physical interactions between individual microgels are typically weaker than the covalent bonds within them. This allows the microgels to flow when external forces, such as those applied during printing, overcome interparticle friction, resulting in superior shear-thinning properties [71]. Fang et al. [104] fabricated gelatin methacryloyl (GelMA) microgels using a microfluidic method, and used these to prepare two types of GelMA microgel-based bioinks: microgel-based biphasic (MB) bioink and jammed microgel (JM) bioink. The MB bioink was made by mixing the GelMA microgels with GelMA precursor solution, while the JM bioink was formed by jamming the GelMA microgels. Pure GelMA precursor solution was used to make a control hydrogel-based bioink. The rheological properties of these three types of bioink were compared, as shown in Fig. 3A. The viscosity of all three bioinks decreased with increasing shear rate, demonstrating effective shear-thinning behavior. Notably, both MB and JM microgel-based bioinks exhibited more rapid viscosity reduction compared to the bulk hydrogel bioink, indicating superior shearing-thinning performance. Kessel et al. [96] fabricated high-aspect-ratio hydrogel micro-strands by deconstructing a bulk hydrogel through a grid with apertures of 40 and 100 μm. As shown in Fig. 3B, entangled micro-strands obtained with both 40 μm (i) and 100 μm (ii) apertures displayed shear thinning behavior, which could be adjusted by varying the degree of micro-strand crosslinking. Jeon et al. [105] developed a granular microgel bioink by mixing human mesenchymal stem cells (hMSCs)-laden oxidized and methacrylated alginate (OMA) microgels with DMEM containing 0.05 w/v % photoinitiator. The hMSC-laden OMA microgel beads were directly assembled into well-defined, complicated 3D shapes and structures via photopolymerization. The shear-thinning behavior of this bioink was confirmed by rheological testing, indicated by viscosity decreases against the increased shear rate (Fig. 3C(i)) and shear strain (Fig. 3C(ii)).

4.3.2. *Yield stress*

Yield Stress of a hydrogel is the minimum stress required to initiate flow, typically arising from intermolecular interactions within polymer chains [107]. This leads to the creation of a delicate, physically crosslinked network that can be disrupted by shear forces beyond the yield stress threshold, and the network undergoes gradual reformation when the shear force is removed. In contrast to high viscosity, which primarily delays the collapse of a fabricated 3D structure, the presence of yield stress can potentially hinder both flow initiation and collapse [19]. Flégeau et al. fabricated tyramine-modified hyaluronic acid (HA-TYR) microgels by mechanically sizing bulk HA-TYR bulky hydrogels through meshes and mixed the microgels with 1 % unmodified HA and human

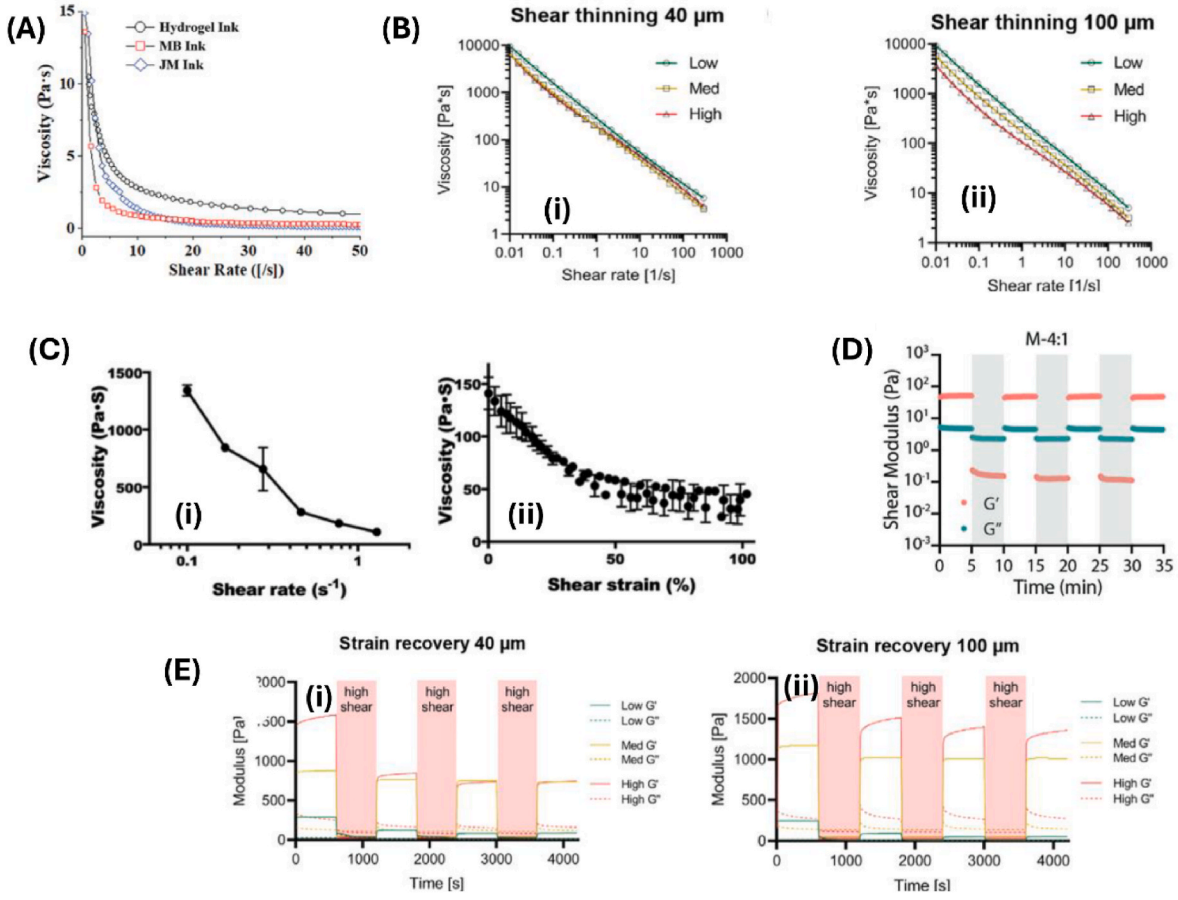


Fig. 3. The rheological properties of microgel-based bioinks and the difference between microgel-based bioinks and hydrogel-based bioinks. (A) Different shear-thinning behavior of GelMA hydrogel bioink, GelMA microgel-based biphasic (MB) bioink, and jammed microgel (JM) bioink. Reproduced with permission [104]. Copyright, 2021 Wiley-VCH GmbH. (B) (i) Shear thinning behavior of entangled micro-strands with 40 μm apertures at low, medium, and high crosslinking degrees. (ii) Shear thinning behavior of entangled micro-strands with 100 μm apertures at the same crosslinking levels. Reproduced under the terms of the Creative Commons CC-BY license [96]. Copyright 2020, The Authors, Published by WILEY-VCH GmbH. (C) (i) Shear rate, and (ii) shear strain demonstrate the shear-thinning behavior of OMA microgel-based bioinks loaded with hMSCs Reproduced with permission [105]. Copyright 2018, The Authors, published by Elsevier. (D) Shear recovery and time sweep of microgel-templated porogel (MTP) microgel bioinks. Reproduced with permission [106]. Copyright 2021, WILEY-VCH GmbH. (E) (i) Shear recovery behavior of entangled micro-strands with 40 μm apertures at low, medium, and high crosslinking degrees. (ii) Shear recovery behavior of entangled micro-strands with 100 μm apertures at low, medium, and high crosslinking degrees. Reproduced under the terms of the Creative Commons CC-BY license [96]. Copyright 2020, The Authors, Published by WILEY-VCH GmbH.

chondrocytes to form the microgel bioink [108]. The bioinks containing different diameter microgels had consistent yield stress of 139 Pa, indicating that the friction forces between microgels were not influenced by granule size. All bioinks exhibited shear-thinning behavior, with a linear relationship between viscosity and shear rate. Oscillatory strain sweeps showed that the bioinks transitioned from a solid to liquid-like state under high shear stress, with excellent shear recovery properties upon returning to low shear conditions. Printing experiments with different aperture diameters confirmed that the HA-TYR bioinks could be sed to print structures with good shape recovery and resolution.

4.3.3. Shear recovery

Shear recovery refers to the capacity of a material to regain its original structure after exposure to high shear rates, assessed by measuring the change in viscosity over time. This involves initial viscosity measurement, followed by a period of high shear to disrupt the microstructure, and subsequent reduction of applied forces to monitor material recovery from shear impact [109]. Microgel bioinks are likely to show better shear-thinning and shear recovery properties compared to bulk hydrogel bioinks, since they comprise discrete, micron-sized gel particles suspended in a liquid medium. Under shear stress, such as during extrusion bioprinting, the weak physical interactions between the particles cause rapid reduction in viscosity, allowing smooth flow through the nozzle. Once shear stress is removed, microgels can rapidly recover their shape due to their flexible, crosslinked polymer network and the low friction between particles. This quick recovery enhances the shape fidelity and structural integrity of the printed construct. In contrast, conventional bulk hydrogel-based bioinks consist of continuous polymer networks that typically exhibit higher initial viscosity and less pronounced shear-thinning behavior compared to microgels. This increased viscosity can make extrusion more challenging, leading to a higher risk of clogging or the need for greater extrusion pressures, which may negatively impact cell viability.

Due to their particulate nature, microgel bioinks can provide increased control over print fidelity and higher resolution. Their rapid viscosity recovery after extrusion helps preserve the printed shape and structure, minimizing issues such as sagging or spreading. For conventional bulk hydrogel-based bioinks, it is more challenging to maintain high print fidelity and structural precision due to increased flow in the continuous hydrogel network after printing. The enhanced shear-thinning and rapid recovery properties of microgel-based bioinks can also improve biological interactions. For example, cell viability during printing can be better preserved due to reduced mechanical stress on cells. Moreover, the discrete nature of microgels can allow better distribution and encapsulation of cells within the bioink. In comparison, ensuring even cell distribution within a bulk hydrogel matrix is more challenging [110,111]. Higher extrusion pressures and the continuous network structure can also impose more stress on cells during printing, potentially reducing cell viability. Ouyang et al. [106] developed microgel-templated porogel (MTP) bioinks by adding gelatin microgels into a GelMA solution. The gelatin microgels acted as templates, which were removed by melting over time when the matrix was heated at 37 °C, creating controlled microporosity in the printed hydrogels. Rheological tests confirmed that at a 4:1 microgel-to-matrix volume ratio, the bioink showed complete recovery of G' and G" under high (300 %) (grey shadow) and low (1 %) (plain) strain cyclic sweeps (Fig. 3D), indicating a fast sol-gel transition. As previously mentioned, Kessel et al. [96] produced entangled micro-strands, where those made with 40 μm (Fig. 3E(i)) and 100 μm (Fig. 3E(ii)) apertures were shown to have good shear recovery behavior when subjected to repeated cycles of low and high shear.

4.4. Functionality

The functionality of a bioink refers to its ability to effectively support the bioprinting process and the subsequent biological activities necessary for creating viable tissue constructs. Ideally, bioinks should closely mimic the composition of native tissue ECM and be amenable to use with different printing technologies. They should be designed with fundamental properties to meet various complex requirements for tissue regeneration and perform the desired biological functions [112,113]. Bioink functionality is dependent on several key properties, including biocompatibility, microporosity, shape fidelity, tunability, and heterogeneity [112,114].

4.4.1. Biocompatibility

Biocompatibility of a bioink refers to its ability to interact safely with biological systems without causing adverse reactions that could impact the acceptance and long-term performance of the bioprinted construct in the body [115]. Biocompatibility is a fundamental requirement for bioinks, crucial for supporting cell growth and tissue regeneration. Specifically, bioink needs to ensure high cell viability during and after the printing process, avoiding toxic components and harsh crosslinking conditions. It should also facilitate cell adhesion, spreading, and interactions with the bioink matrix, and enable sufficient diffusion of nutrients and oxygen to support cell survival and function. Although both conventional hydrogel-based and microgel-based bioinks are typically derived from widely used biocompatible materials free of toxic components, differences in their printability can influence how the printing process affects the viability of cells embedded within the bioinks.

During the printing process, cell injury can arise from various factors, including shear stress, thermal stress, and radiative stress. The extent of injury depends on the strength and duration of these stresses, with excessive forces beyond cellular tolerance potentially causing irreversible damage and unintended apoptosis [116]. To ensure cell viability and bioactivity of the printed structure, specific conditions need to be maintained during bioprinting including temperature, pH, pressure, physical forces, and osmolarity. Due to these stringent requirements, a universally optimal hydrogel-based bioink for 3D bioprinting has not yet been developed [113]. Although comparative studies are limited, microgel-based bioinks can be expected to offer better cell protection than traditional hydrogel-based bioinks. In microgel-based bioinks, cells can be encapsulated within individual microgels and hence become better protected from the stresses of the printing process. This is augmented by their better shear-thinning behavior compared to hydrogel-based bioinks, further reducing the shear stresses experienced by cells during extrusion [88,116]. Their unique cell encapsulation ability also allows microgel-based bioinks to achieve a more homogeneous cell distribution, avoiding cell clumping and ensuring uniform cell growth, leading to improved tissue regeneration [17].

4.4.2. Microporosity

Microporosity refers to the presence of microscale pores or voids within the bioink structure. Microgel-based bioinks contain abundant void spaces between microgels, forming interconnected micropores that enhance mass transport and distinguishing them from bulk hydrogels [24,29]. They are hence useful for constructing biomimetic scaffolds possessing multiscale pore structure. Scaffolds printed using conventional hydrogel-based bioinks lack microporosity due to having nanoscale voids between crosslinked polymer chains, which can reduce cell viability, limit cell migration or the infiltration of endogenous cells and surrounding tissues and impede the exchange of nutrients and metabolic waste [117]. These factors can result in poor implant-host integration and affect the degradation of printed hydrogel constructs [17]. In contrast, the size of microgels and their assembly methods create a microporous structure in the printed constructs, significantly improving the transport of nutrients, oxygen, and metabolic waste, as well as cell viability and cell-cell interactions [77,79,96].

Shao et al. [117] used microgels as sacrificial templates within a GelMA bioink, resulting in printing structures with mesoscale pore networks (MPNs) that could greatly enhance nutrient delivery and cell

growth. Fig. 4A(i) illustrates the process of 3D bioprinting constructs with macroporous networks. The sacrificial bioink, composed of a cell/GelMA mixture and gelled gelatin microgels, is first thermo-crosslinked to create temporary, predesigned cell-laden structures through extrusion bioprinting onto a chilled platform. The microporous structure could be controlled by various parameters such as changing the extrusion nozzles used for making gelatin microgels, and the volume of microgels in the bioink. Constructs with dimensions of 10 × 15 mm could be printed with the bioink and microporous structure with pores size of 300–500 μm could be observed in the vertical section of the bioprinted structures. When MSC3T3-E1 cells and human umbilical vein endothelial cells (HUVECs) were cultured in the constructs, confocal laser scanning microscopy (CLSM) images show the spreading of MC3T3-E1 cells and HUVECs encapsulated within the constructs (Fig. 4A(ii)).

Along these lines, Feng et al. prepared microgels using droplet-based microfluidics, where phenylboric acid groups were introduced into a methacrylate hyaluronic acid (HAMA) backbone and crosslinked with gelatin methacrylate (GelMA) [118]. These microgels were then made into a dynamic cross-linked microgel assembly (DC-MA) bioink with dopamine-modified hyaluronic acid as a dynamic cross-linker. The DC-MA bioink exhibited stable microporosity of approximately 33.3 % in repeated trials, indicating a robust internal structure (Fig. 4B(i)). In the dye-diffusion test, red dye was shown to rapidly permeate the DC-MA bioink, whereas no significant dye penetration was observed in the bulk hydrogel (Fig. 4B(ii)). In addition, the microporous structure of the construct made of DC-MA bioink significantly enhance the infiltration of L929 cells seeded on the surface of the hydrogel and DC-MA constructs on day 1 and day 7 (Fig. 4B(iii)). This difference was attributed to the abundant interconnected micropores within the construct made of DC-MA bioink. Our previous studies also showed that microgel assemblies derived from microgel fibers could facilitate much faster dye transfer compared to bulk hydrogels due to their highly interconnected microporous network [79]. Such findings suggest that a highly interconnected microporous structure within the bioink is key to enabling rapid nutrient exchange and mass transport in bioprinted scaffolds.

4.4.3. Shape fidelity

Shape fidelity refers to the accuracy with which the bioink can replicate and maintain the intended shape and structure of the bioprinted construct during and after the printing process [36]. This quality is critical for ensuring a close match of the printed construct with its digital design and intended function, especially for tissue engineering applications. Shape fidelity encompasses several aspects, including the shape retention of single filaments upon extrusion, and the extent of similarity between the printed construct and the original computer design [119]. The shape fidelity of a single extruded filament is reflected by how closely its cross-sectional shape matches a perfect circle (filament circularity). High filament circularity ensures that the printed lines are uniform in thickness and consistent in shape, which is crucial for achieving the desired geometric precision and structural integrity of the final construct. The shape fidelity of a whole printed construct is reflected by the degree of similarity between the actual construct and its intended design, including the match in dimensions, geometry, and features as specified in the digital model.

Traditional hydrogel-based bioinks, characterized by their homogeneous and continuous polymer networks, are more likely to collapse under their own weight during layering. They are also prone to deformation or sagging prior to crosslinking and often undergo shrinkage or distortion during the crosslinking or stabilization process, all of which compromise shape fidelity [16]. Microgel-based bioinks may exhibit superior shape fidelity due to their unique structural and mechanical properties, including enhanced shear-thinning and recovery behavior,

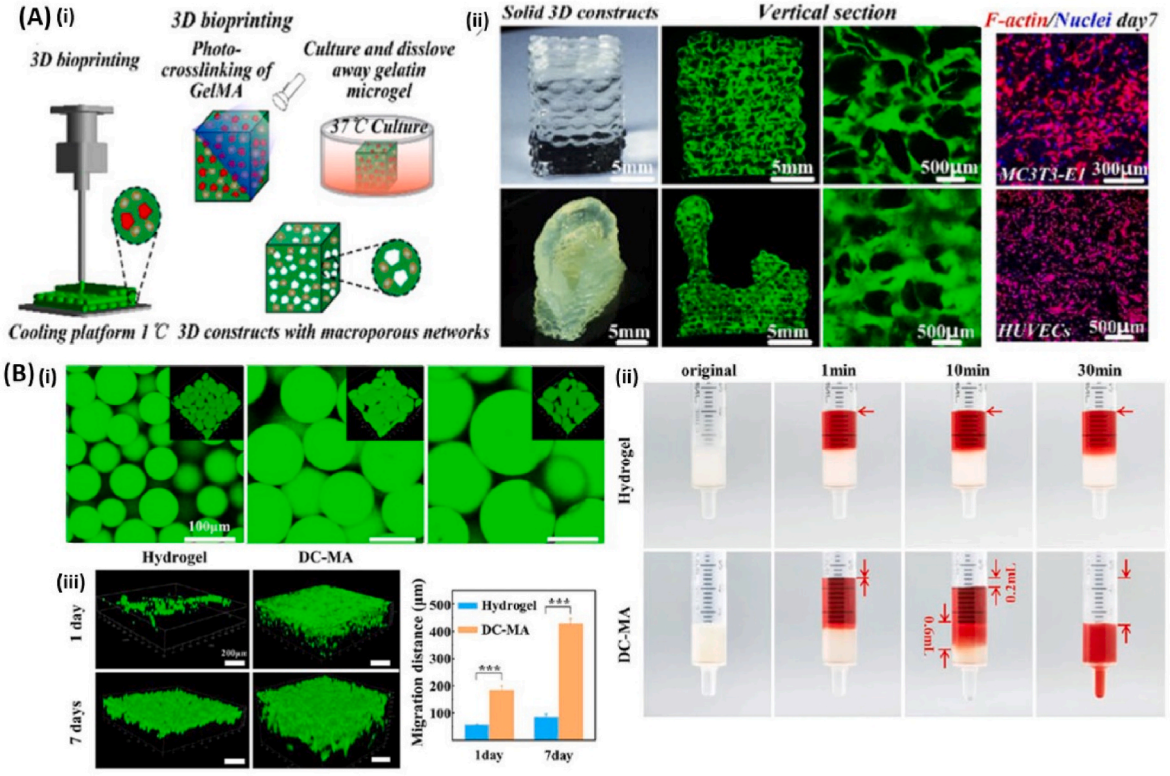


Fig. 4. Microporosity of microgel-based bioinks. (A) (i) The process of 3D bioprinting constructs with MPNs with 3D printed constructs with macroporous networks. (ii) Digital images and confocal laser scanning microscopy (CLSM) images of bioprinted structures. Reproduced with permission [117]. Copyright 2020, Zhejiang University Press. (B) (i) Microporous structure of the construct made of DC-MA bioink (ii) Dye diffusion test result. (iii) Cell infiltration behavior of hydrogel and constructs made of DC-MA bioink at Da y 1 and Day 7. Reproduced under the terms of the Creative Commons CC-BY-NC-ND license [118].

flow control, cross-linking and mechanical strength, as well as the ability to enable higher packing density and reduce shrinkage or deformation. Enhanced shear-thinning behavior facilitates smoother extrusion through the nozzle during printing and allows for rapid recovery once deposited [118], enabling the printed construct to maintain its shape immediately after extrusion. Furthermore, the close packing of microgel particles [18] improves flow control during extrusion, enhances mechanical stability, increases packing density, and reduces shrinkage or deformation during post-printing processes [78]. These characteristics significantly enhance shape fidelity, especially in complex or multilayered scaffold geometries.

Xin et al. developed a polyethylene glycol (PEG) microgel bioink and printed various scaffolds with this bioink using extrusion-printed technique [120]. Scaffold designs were loaded into the bioprinter to create structures with layer height of 500 μm and layer width of 600 μm, using a print speed of 10 mm/s (0.27 mL/min). Two nozzle sizes were tested (840 and 600 μm), where extrusion through the larger nozzle led to continuous, uniform microgel lines while the smaller nozzle resulted in uneven extrusion (Fig. 5A(a)). Diverse shapes were printed, including a 3 cm diameter honeycomb (b, c and e), and a hollow 2 cm tall cylindrical structure (d-f). 2D printing of a three-layer honeycomb structure confirmed the ability for continuous bioink extrusion to form a coherent structure while 3D printing of a cylindrical structure demonstrated exceptional Z-axis stability, supporting a 2 cm height without collapse, which surpassed prior bioprinting attempts using non-viscous materials. The structures remained robust even when tilted at 90°, underscoring robust microgel adhesion (Fig. 5A(f)). A nose and an ear were printed with the PEG microgel-based bioink and the PEG microgels exhibited good shape fidelity as indicated by negligible differences between the designed and actual values of the construct dimensions (Fig. 5B). The ear model was designed to have a dimension of 4.15 × 2.85 × 0.719 cm, with a helix of 1.35 cm and a canal of 0.46 cm while the printed ear shape had a dimension of 4 × 2.72 × 0.7 cm, with a helix of 1.31 cm, and a canal of 0.41 cm. Similarly, the nose model was designed to have a dimensions of 3.5 × 2.49 × 1.48 cm, with a nostril of 0.42 cm while the printed nose shape had a dimension of 3.5 × 2.3 × 1.4 cm, with a nostril of 0.42 cm. The MB bioink also enabled the successful 3D printing of various other models (Fig. 5C). All these results indicate the good shape fidelity of the MB bioink.

As previously mentioned, Fang et al. [104] developed a GelMA-based biphasic (MB) bioink as shown in Fig. 5C. This bioink demonstrated superior 3D printing capability and shape fidelity, forming uniform filaments following extrusion from the nozzle (a(i)). After labelling the gelatin microgels with red fluorescence and the GelMA solution with green fluorescence in the MB bioink, the microgels (red) were observed to be evenly extruded with interstitial hydrogel precursor (green) (a (ii)). The MB bioink was used with extrusion bioprinting to create simple structures, including a mesh (b(i)), double ring (c(i)), tube (d), and hemisphere (f). In the printed structures, the junctions of two perpendicular filaments displayed good interconnectivity between adjacent layers as shown through microscopy (b (ii)). The double-ring structure was printed with rhodamine-conjugated GelMA MB bioink to visualize the granular morphology of the printed fibers (c(i)). The MB bioink showed temperature-independent rheological properties, and its printing fidelity was not affected by changing the nozzle temperature during printing from 15 to 30 °C. This consistency can be highly advantageous for multi-material printing and bioprinting in extreme environmental conditions with temperature instability. The geometry of the printed tubular structure was measured (d), showing good shape fidelity with a marginal reduction of less than 5 % attributed to the width of the filament building blocks (e). The printed hemisphere exhibited a smoother interlayer transition (f) and dimensions closer to the input values, suggesting high structural fidelity imparted by the MB bioink (g).

Excellent extrusion consistency and shape fidelity were also obtained when the MB bioink was used to print complicated structures resembling human anatomical components, such as a large and thick vessel (h), meniscus (i), nose (j), ear (k), bronchus (l), and brain model (m-o). For example, the bronchus (l) was printed with a high aspect ratio geometry (15 mm height, 5 mm interior diameter) and very thin walls (1 mm thickness), and provided excellent support for its own weight during printing. Using suspension printing, the MB bioink was used to fabricate a brain model (m) displaying typical folded and wrinkled areas on the surface (n). Comparison between the geometry of the template (grey) and the printed structures (red and orange) showed that 94.6 % of voxels in the printed structure were within 0.5 mm of the template (o), confirming a high level of printing fidelity.

4.4.4. Tunability

The tunability of microgels refers to the ability to customize their physical, chemical, and mechanical properties [121]. Microgel properties can be tuned by changing their shape, size, stiffness, composition, packing density and inter-particle density [85,88], as shown in Table 2. The tunability of microgels and hence microgel-based bioinks is greatly advantageous for 3D bioprinting. Microgels with various morphologies have been developed, such as microspheres (Fig. 6A), microfibers (Fig. 6B), irregular particulates (Fig. 6C), microrods (Fig. 6D) and microribbons [20,58,122–124], as illustrated in Fig. 6. The aspect ratio of microgels (the ratio of the longest to the shortest dimension) can

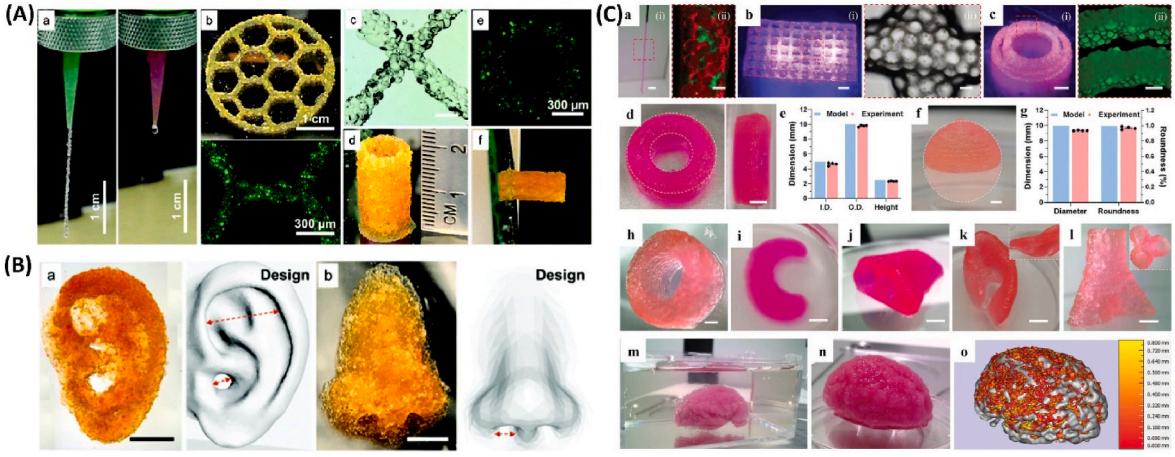
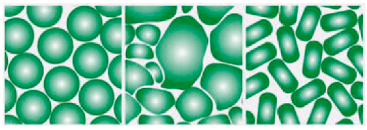
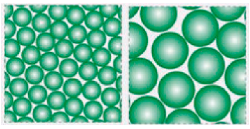
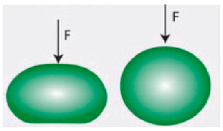
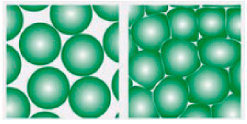


Fig. 5. Shape fidelity of microgel-based bioink. (A) Diverse shapes were printed using PEG microgel bioink. (B) The PEG microgel bioink has good shape fidelity. Reproduced with permission [120]. Copyright 2019, The Royal Society of Chemistry. (C) 3D printing capability and fidelity of MB bioink. Reproduced with permission [104]. Copyright, 2021 Wiley-VCH GmbH.

Table 2
Tunability of microgel. *Adapted from* Ref. [88].

Characteristics	Design	Description
Shape		Spherical, Irregular, Rod
Size		Small or Large as per application
Stiffness		Soft or Stiff
Composition		Uniform or Heterogenous
Packing Density		Low or High
Interparticle crosslinks		Covalent or Dynamic

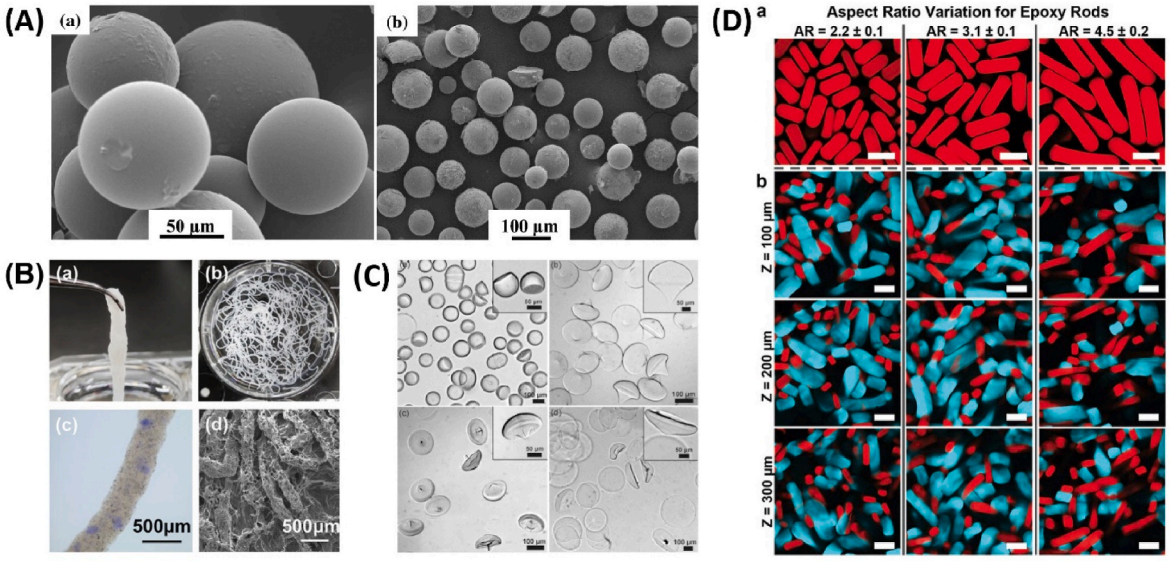


Fig. 6. Microgels Synthesized in Various Shapes (A) Microspheres gels. Reproduced with permission [130]. Copyright 2022, The Authors, under exclusive license to Springer Science Business Media, LLC, part of Springer Nature. (B) Microfiber gels. Reproduced under the terms of the Creative Commons CC-BY license [79]. Copyright 2023, The Authors, Published by WILEY-VCH GmbH. (C) Irregular particulate gels. Reproduced under the terms of the Creative Commons CC-BY license [20]. Copyright 2012, The Authors, published by AIP Publishing. (D) Microrod gels with different aspect ratios. Reproduced under the terms of the Creative Commons CC-BY license [131]. Copyright 2022, The Authors, Published by WILEY-VCH GmbH.

significantly influence the properties of printed scaffolds [125]. Spherical microgels typically have an aspect ratio of around 1, while low-aspect-ratio (1–5) and high-aspect-ratio (>5) microgels have been developed in various studies to enhance tissue regeneration [58]. By printing high-aspect-ratio microgels, such as micro-strand gels and microfiber gels, constructs with aligned gels can be fabricated to direct cell alignment and subsequent differentiation in the regeneration of specific tissue types [96]. In addition to shape and geometry, microgels can be tuned for stiffness and overall mechanical strength, as well as degradation behavior by adjusting crosslinking density, polymer composition, and molecular weight. This tunability is particularly important in 3D bioprinting for ensuring shape fidelity and scaffold stability. Moreover, microgels can be easily engineered to respond to environmental stimuli such as temperature, pH, ionic strength, and solvents, making them a useful component of responsive bioink materials for creating drug delivery systems or smart scaffolds in tissue engineering [126]. The surface of microgels can also be modified with functional groups, ligands, or biomolecules to enhance their interaction with cells, drugs, or other materials [85]. Additional tunability arises from customizing microgel networks to achieve a desired porosity or pore structure, useful for creating bioinks that can promote cell adhesion and proliferation, tissue integration, and therapeutic delivery.

In contrast, hydrogel-based bioinks possess limited tunability. The printability and functionality of a hydrogel-based bioink depend heavily on the composition and properties of the hydrogel precursor solution, as well as crosslinking density and uniformity [127]. For example, their low mechanical stability and unpredictable degradation limit the effectiveness of hydrogel-based bioinks for printing scaffolds that are viable in long-term cell culture or *in vivo* applications [128]. Many studies have hence added micro- and nanofillers into bulk hydrogel-based bioinks to improve electrical conductivity, mechanical properties, cell-matrix interactions, and cell maturation in the printed scaffolds, and facilitate the design of stimuli-responsive constructs [129]. However, the problem of nanoporous structure in bulk hydrogels are not addressed by these approaches, and the use of fillers constituting a different phase within the hydrogel raise potential long-term concerns associated with safety, metabolism, and biodegradation [44,129].

4.4.5. Heterogeneity

Natural tissues are highly heterogeneous, composed of different cell types, ECM components, and biochemical cues. Bioink heterogeneity is therefore an important characteristic for creating functional and durable bioprinted constructs that faithfully replicate the complex and multifaceted nature of native tissues, as well as for enhancing cell differentiation, tissue integration, mechanical performance, and nutrient exchange [129,132]. To create heterogeneity in bioprinted scaffolds using hydrogel-based bioinks, such as variations in mechanical properties, cell distribution, and biochemical cues, a variety of strategies have been tried including multi-material printing, coaxial bioprinting, sequential layering, and post-printing functionalization [133,134]. However, these strategies are not easily realized as they each come with technical limitations related to bioink complexity, material compatibility, scalability, and technical precision. Balancing these constraints with the desired tissue characteristics is key to advancing the development of heterogeneous bioinks in 3D bioprinting.

In comparison, achieving heterogeneity is notably more straightforward with microgel-based bioinks due to the inherent modularity, flexibility, and controllability of microgels [135]. The modularity of microgels arises from their morphology as small, discrete particles that can be individually tuned in properties, enabling mix and match of microgels to create a heterogeneous environment. The ability to pre-tune and combine microgel particles with different properties allows for improved spatial control over mechanical, biochemical, and cellular environments, ultimately simplifying the creation of complex, tissue-like structures. Fig. 7 shows the strategies to achieve various forms of heterogeneity. For example, microgels with different mechanical properties (high and low stiffness) can be mixed in the same bioink to create mechanical heterogeneity (A), in turn leading to anisotropic cell growth, proliferation, and differentiation in the printed construct. Sacrificial and non-sacrificial microgels can be combined to achieve heterogeneity in microporous structure (B). Heterogeneity in bioactivity can be achieved by embedding different growth factors (C) or cell types (D) into microgels within the same bioink. A combination of these strategies can lead to advanced techniques for creating tissue-like spatial heterogeneity in a unified bioink system that is necessary for functional tissue regeneration (E) [75].

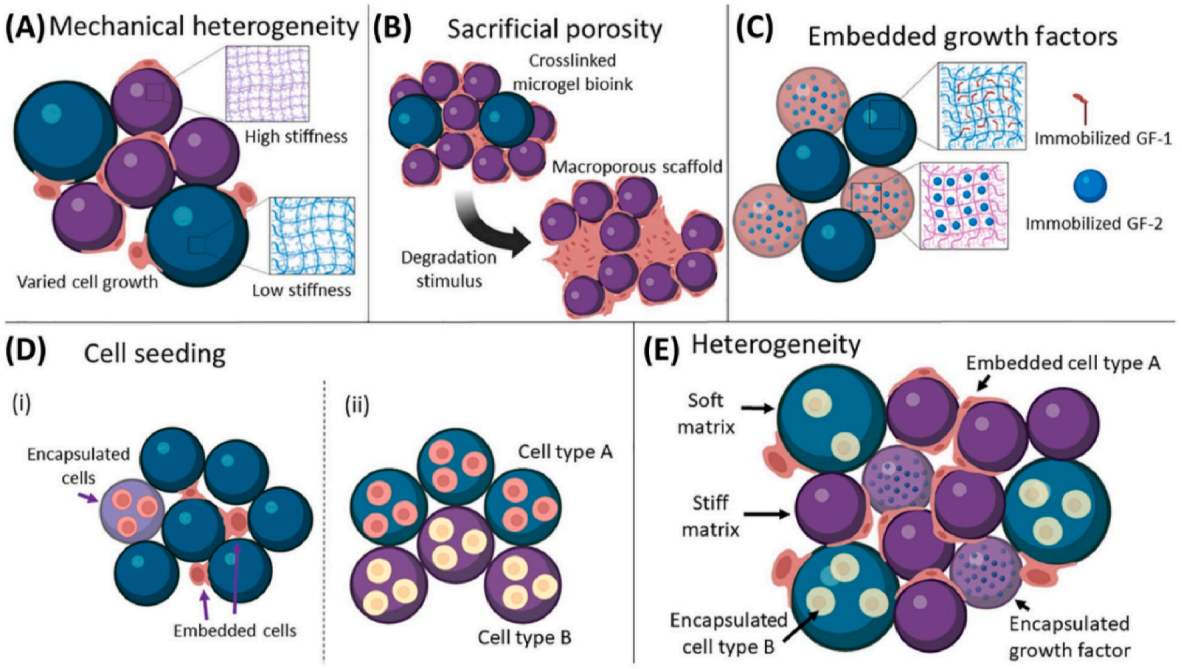


Fig. 7. The strategies to achieve heterogenicity of microgel-based bioinks. Reproduced with permission [75]. Copyright 2023, published by Elsevier Ltd.

4.5. Comparison between hydrogel and microgel-based bioinks

In summary, hydrogel-based bioink mainly comprises hydrogel precursor solution where polymer chains flow in solvent (usually water), while microgel-based bioink is mainly composed of microgel particles. The difference in composition significantly influences their printability and functionality, as summarized in Table 3.

5. Fabrication of microgel-based bioinks

Various materials, including naturally derived and synthetic polymers have been used to produce microgels through different techniques, such as batch emulsion, microfluidic emulsion, lithography, electrohydrodynamic spraying, and mechanical fragmentation [21,136]. The synthesis and properties of microgels have been discussed in several reviews, including the materials and techniques used [21,24,58,136–139]. However, to apply them as a bioink, there are specific requirements placed on microgel properties which are not well captured in the literature, such as size, materials selection, and rheological properties including viscosity and shear thinning. This section discusses the materials and methods relevant for synthesizing microgels with the intention to use them as bioinks.

5.1. Materials for producing microgels for use as bioinks

The formulation of microgel bioink plays a pivotal role in the success of bioprinting approaches for tissue regeneration. The rational selection and characterization of constituent materials for the microgel bioink is an essential step prior to scaffold fabrication by 3D bioprinting and should consider the printing modality as well as intended application. Microgels can be synthesized with the same materials that have been used to make bulk hydrogels, including natural or synthetic biomaterials and their combinations. Here, we present a summary of materials that have been used specifically for producing microgel bioinks for extrusion-based bioprinting, as shown in Table 4.

5.2. Methods for producing microgels

Several methods can be used to produce microgels, such as batch emulsion, microfluidic emulsion, mechanical fragmentation, photolithography, and electrodynamic spraying, which have been summarized in recent reviews by our group [58] and others [149]. Briefly, batch emulsion combines immiscible oil and aqueous hydrogel precursor solutions, resulting in droplet formation through external mechanical energies delivered by shearing, supersonic, and spraying processes [150]. Meanwhile, microfluidics emulsion produces well-defined channel geometries by using viscous forces, inertial forces, interfacial tension, and in some cases buoyancy, to disperse two or more immiscible phases under continuous flow conditions [151]. Mechanical fragmentation involves the rapid breakdown of initially crosslinked hydrogels into smaller particles through techniques such as homogenization, sonication, and high-pressure microfluidization [149]. Photolithography techniques encompass imprint lithography, where a hydrogel precursor is molded and crosslinked; photolithography, where the precursor is selectively solidified using photomasks; and flow lithography, where the flowing hydrogel precursor is periodically solidified by a light mask, all leading to the formation of microgel particles [152,153]. Electrodynamic spraying works by creating an electric field between a metal needle and a receiving device, allowing droplets to overcome surface tension and be sprayed into the receiving device, where they form microgels after crosslinking [154].

Theoretically, all of these methods produce microgels that can later be used to formulate bioinks. However, bioprinting introduces various requirements on microgel size and rheological properties, making some methods more suitable than others for synthesizing microgels with intended use as bioinks. Batch emulsion, microfluidic emulsion, and mechanical fragmentation are more widely used to produce microgels with suitable properties for 3D bioprinting, providing the benefits of scalability, precision, and simplicity. Table 5 summarizes the methods used to produce microgels with their respective advantages and disadvantages, the geometry of the resulting microgels, and whether the microgels have been applied as bioink materials.

Table 3
Differences between key properties of hydrogel-based and microgel-based bioinks.

Property	Hydrogel-based Bioink	Microgel-based Bioink
Printability	Narrow biofabrication window	Wide biofabrication window
Biocompatibility	Good	Excellent
Microporosity	No	Excellent
Shape fidelity	Limited	Good
Tunability	Limited	Excellent
Heterogeneity	Limited	Excellent

Table 4
Materials used to synthesize microgels used as bioink materials.

Materials	Resources	Microgel shape
Chitosan [140]	Natural	Microspheres, microfibers [141]
Collagen I [142]	Natural	Microspheres, microfibers
Gelatin [117,143,144]	Natural	Microspheres, microfibers [145]
Fibrin [146]	Natural	Microfibers [146]
Hyaluronic acid (HA) [108]	Natural	Microspheres, microrods [125]
Alginate [99]	Natural	Mushroom-like, hemi-spherical [20]
Polycaprolactone (PCL) [147]	Synthetic	Microspheres, microcylinders [148]

6. Jamming phenomenon of microgel-based bioinks during bioprinting

The jamming phenomenon of microgel-based bioinks in 3D bioprinting refers to a state where microgel particles within the bioink become densely packed, resulting in a transition from fluid-like to solid-like behavior until an external force is applied with sufficient strength to initiate movement. In the case of highly dense microgel formulations, the microgels can deform elastically under stress up to a certain point, but individual microgels do not move on their own. However, if the applied stress surpasses a critical threshold, the microgels start moving relative to each other as the stress overcomes the forces holding them in place. When the applied stress is reduced below this threshold, the system recovers to its initial state. This ability of the jammed microgel system to flow and recover in response to stress confers inherent printability in 3D printing bioinks, without requiring changes in the molecular structures of the materials being printed. Importantly, the jamming phenomenon of microgels is expected to work independently of their composition, potentially enabling 3D bioprinting of a wide range of hydrogel-forming materials that can be processed into microgels [87]. Thus, the jamming of microgel bioinks is a key factor in 3D bioprinting that influences the printability, structural integrity, and overall performance of the printed constructs. Properly tuned jamming can improve the printability of bioinks by allowing them to flow through the printer nozzle and then solidify quickly to maintain the desired shape and structure. This transition is essential for creating detailed and complex structures. Moreover, the jamming state can influence the encapsulation and distribution of cells within the bioink, as well as work in conjunction with gelation and crosslinking processes to maintain the shape or enhance the mechanical properties of the printed construct. Once the bioink is deposited, the densely packed microgels provide mechanical stability, preventing the construct from collapse or deformation.

Fig. 8A illustrates the process by which microgels undergo jamming during their assembly into a bioink. Jamming involves the concerted interactions and arrangements of microgels as they come together to form a cohesive bioink matrix. In a packed or jammed state, microgels physically contact, support, and squeeze each other, appearing like bulk

Table 5
A summary of various techniques used to synthesize microgels that have been incorporated into bioinks.

Technique	Advantages	Disadvantages	Geometry of microgels	Application as bioink material
Batch emulsion	Scalable; Simple process; Suitable for large-scale production	Limited control over microgel geometry and uniformity	Microspheres, irregular shapes [155]	Yes [156]
Microfluidic emulsion	Precise control over microgel geometry and uniformity; High throughput and reproducibility	Specialized equipment and expertise required	Microfibers, microrods [151]	Yes [104,157]
Mechanical fragmentation	Simple process; No specific equipment and expertise required	Limited control over microgel geometry and uniformity; Need bulk hydrogel first	Microrods, microfibers, irregular shapes [149]	Yes [149]
Photolithography	High precision and control over geometry	Complex process; Expensive; Limited scalability	Micropatterns, custom shapes	No
Electrodynamic spraying	Ability to create fine particles; High throughput	Requires specialized equipment; Limited control over geometry	Microspheres, microfibers	No

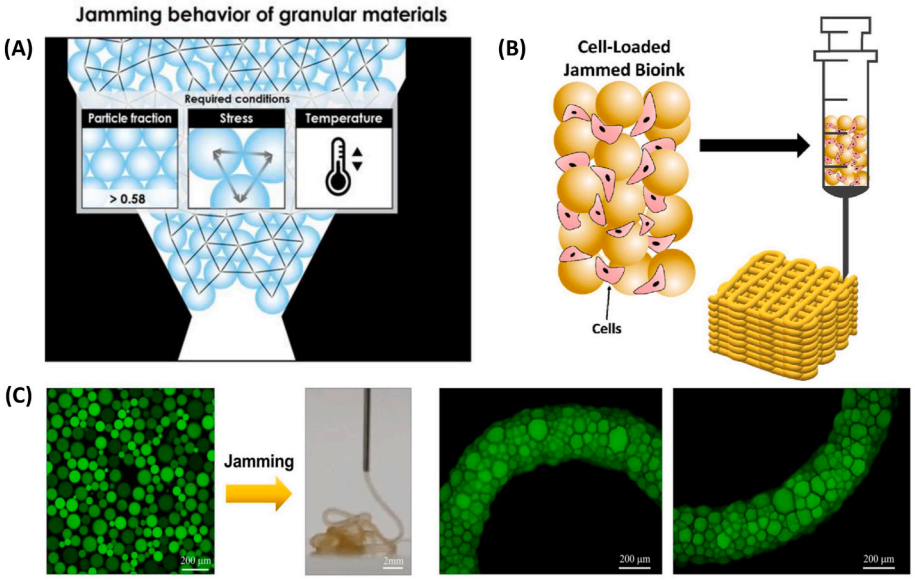


Fig. 8. Jamming of microgel-based bioinks. (A) Schematic of the process by which microgels undergo jamming to assemble into a bioink. Reproduced with permission [76]. Copyright 2018, published by Elsevier Ltd. (B) Schematic of jammed alginate microgel-based bioink loaded with living cells. (C) Microscopy observation of microgels within the jammed microgel-based bioink labelled in green. All images are reproduced with permission [156]. Copyright 2023, published by Elsevier Ltd.

hydrogels. However, since the inter-microgel interactions are much weaker than the covalent bonding inside microgels, the microgel ink can yield to flow when external forces overcome the inter-microgel friction. Once the printing pressure is withdrawn, the physical interactions between microgels can restore them to a quasi-solid state, which confers shear-thinning properties to the microgel bioink. Importantly, microgels remain intact during the entire printing process due to the stable intra-microgel covalent network. This not only protects encapsulated cells from high shear stress but also enhances the printing stability [118]. Lee et al. [156] developed a tyramine-conjugated alginate microgel-based bioink. When this bioink was used in 3D bioprinting, the aqueous media between spherical microgel particles were removed using vacuum filtration, and "jamming" of the cell-loaded microgel-based bioink was formed, as illustrated in Fig. 8B. Fig. 8C further shows the observation of the jammed status of the microgels labelled in green with fluorescein isothiocyanate in a filament during printing. This jamming phenomenon of microgel-based bioink led to excellent printability, due to liquid-like behavior of the bioink during high strain and solid-like behavior during low strain, which improved cell viability and printing resolution [156].

7. Strategies for assembling microgels post-printing

To print constructs with microgel bioinks, individual microgels in the bioink need to be assembled immediately post-printing by leveraging interparticle interactions [126]. Microgel assembly strategies can be categorized by the underlying principles and techniques employed. Several strategies for assembling individual microgels into scaffolds have been summarized in recent reviews, including chemical crosslinking, physical crosslinking, cell-cell interaction assembly, and external force assembly [24,80,110]. Each strategy offers unique advantages and challenges in the context of 3D bioprinting, depending on the desired properties and application of the assembled microgel constructs. The choice of strategy often depends on factors such as the size and composition of microgels, the required precision and stability of the assembly, and the intended application including in drug delivery, tissue engineering, or biosensor development [158]. The advantages and disadvantages of microgel assembly strategies along with corresponding examples of bioinks are listed in Table 6.

7.1. Chemical crosslinking assembly

In microgel assembly by chemical crosslinking, covalent bonds form between microgels through chemical reactions. Methods including enzymatic catalysis, photo-polymerization, and click chemistry can be used to create stable microgel assemblies.

Table 6
Summary of microgel assembly methods in 3D bioprinting.

Method	Advantages	Disadvantages	Microgel bioink example
Chemical Crosslinking			
Enzymatic catalysis	• Gentle conditions • Cytocompatibility	• Fragile enzyme activity • Potential side reactions	• Polyethylene glycol (PEG) [118] • Hyaluronic acid (HA) [158]
Photo-polymerization	• Mild conditions • Short reaction time • High resolution	• Possible cell damage • Incomplete crosslinking	• Alginate [156] • Gelatin [106]
Click chemistry	• Mild conditions • Efficient reactions	• Need complicated functionalization	• PEG [120]
Physical Crosslinking			
Host-guest interaction	• Biocompatible • Spontaneous reactions	• Weak and unstable binding force, especially in aqueous solutions	No examples
Electrostatic Interaction	• Easy-going assembly process and fast reaction	• Easily destroyed electrostatic interaction, especially in electrolyte solutions	• Gelatin [159]
Hydrogen bonding	• Biocompatible and tunable bonding intensity	• Special treatments or complicated fabrication for enhanced hydrogen bonding	• Gelatin [118]
External Driving Force			
Fluidic force	• Simple, fast, and cost-effective	• Complex fabrication, difficulty in forming 3D constructs	• pNIPam [169]
Surface tension	• Easy operation and biocompatibility	• Requires secondary crosslinking for stability, difficulty in forming 3D constructs	No examples

7.1.1. Enzymatic catalysis

Enzymatic catalysis is an emerging and highly effective strategy for assembling microgels into larger, functional structures [159]. As enzymes catalyze the formation of covalent bonds between microgels, this approach leverages the specificity and mild conditions of enzymatic reactions to achieve precise and controlled assembly. So far, transglutaminase [160], horseradish peroxidase [108], laccase [161], and tyrosinase [162] have been used as enzymes to effectively assemble different kinds of microgels. The high specificity of enzymes for their substrates allows targeted assembly and minimal side reactions. In addition, enzymatic reactions typically occur under physiological conditions, such as neutral pH and moderate temperatures, hence helping to preserve the integrity of sensitive cells and biomolecules in the assembled microgel constructs

7.1.2. Photo-polymerization

Photo-polymerization uses photo-initiators and photo-reactive polymers to create photoinduced covalent bonds, by adjusting parameters such as the light source, light intensity, exposure time, and area of illumination. Depending on the viscosity of the biopolymers used, photo-crosslinking can occur before, during, or after the bioprinting process. Typically, low-viscosity polymers are crosslinked before or during printing, while high-viscosity polymers are crosslinked after printing. UV light and visible light have been used for photo-crosslinking to create microgel assemblies in 3D bioprinting [163]. Combination of variations in these parameters allow the fabrication of 3D models that replicate the complexity of both normal and pathological tissues.

7.1.3. Click chemistry

Click chemistry, inspired by natural synthesis of biological molecules, emphasizes efficient, spontaneous, selective, and modular chemical reactions to rapidly form various molecules by combining small units. This technique uses active reactants to form C–X–C bonds under mild conditions, offering advantages such as accessible raw materials, tolerance to water and oxygen, rapid reaction rates, easy product separation, and high yield [164,165].

7.2. Physical crosslink assembly

In microgel assembly by physical crosslinking, microgels can undergo reversible and responsive assembly through non-covalent interactions. Methods such as host-guest interaction, electrostatic interaction, and hydrogen bonding have been used.

7.2.1. Host-guest interaction

Bioinks made of polymers can be physically crosslinked through non-covalent mutual molecular recognition located on different microgel surfaces. Host-guest recognition is aided by hydrophobic interactions, π–π stacking, dipole-dipole interactions, and β-sheet mediated crosslinking. Self-assembling peptides and peptide DNA conjunction are also emerging candidates for crosslinking methods [166].

7.2.2. Electrostatic interaction

Microgel assembly involving the attraction forces exerted by oppositely charged microgels can facilitate interaction-driven microgel organization into structured patterns. This strategy may be more friendly to cells, as ionic crosslinking can be achieved by electrostatic binding of ionic groups in the backbone of polymer chains [166,167].

7.2.3. Hydrogen bonding

Hydrogen bonding occurs when strong electronegative atoms are present on both sides of a hydrogen atom, with one atom covalently bonded to the hydrogen. While an individual hydrogen bond is weak, numerous hydrogen bonds within a microgel system can result in a structure with high mechanical strength [24,168].

7.3. External driving force

Using external forces for microgel assembly eliminates the need for complex material synthesis, where fluidic force, surface tension, and magnetic or acoustic forces may be utilized [24]. These methods can offer quick, simple, and cost-effective assembly, but typically produce unstable structures that require secondary crosslinking for stabilization once the external forces are removed.

7.3.1. Fluidic force

Microfluidic assembly involves using fluidic forces to guide microgels with complementary structures through 1D and 2D railed microfluidic channels, resulting in the creation of complex geometries [24]. This method can allow heterogeneous assembly of microgels made from different types of polymeric materials, eliminating the need for more complex processes to achieve alignment and patterning of different materials. However, to create heterogeneous features, it may be necessary to instead increase the complexity of microgel fabrication. Moreover, the use of microfluidics makes it difficult to produce 3D constructs.

7.3.2. Surface tension

Surface tension assembles microgels by stacking them closely at the

liquid-liquid or liquid-gas interface, where the assembly process is completed by removing the oil or liquid phase. While this method is quick and simple, the resulting microgels are unstable and lack precise size control.

7.3.3. Magnetic forces

Microgel assembly in a magnetic field can occur through three methods: incorporating magnetic nanoparticles into microgels, enhancing the paramagnetic properties of microgels, and using magnetic micro-robots [170]. These approaches leverage magnetic forces to influence microgel formation and behavior.

8. Applications of microgel-based bioink in tissue engineering

Microgel-based bioinks have emerged as an attractive alternative to conventional hydrogel-based bioinks, providing a potentially revolutionary approach for printing complex 3D models to enable targeted tissue regeneration, including skin [171], lung [67,172], heart [88], ear [78], hand [17], bone [78] and skull [78]. Although researchers have achieved success in printing 3D shapes of various organs, the ability to fabricate them with functional capabilities has been largely confined to specific areas within tissue engineering. Limited studies have reported the use of microgel-based bioinks for producing 3D bioprinted constructs for tissue regeneration applications, which have been summarized here.

8.1. Neural tissue constructs printed with microgels bioinks

Nerve injuries can lead to significant functional deficits in patients and increase the risk of secondary injuries or lifelong disability [173]. Clinical treatments rely on pharmacological approaches to reduce the secondary effects of injury, or autologous nerve grafts in cases where the injury creates large gaps in neural connection. However, most treatments are not curative and lead to limited functional recovery in patients, partly due to the complex pathophysiology and inflammatory environment of nerve injuries. Neural tissue engineering has introduced various approaches for fabricating synthetic nerve grafts and neural scaffolds to encourage nerve regeneration [174]. Although different techniques have been attempted to develop scaffolds that mimic the hierarchically aligned structure of native nerve tissue, it remains difficult to accurately replicate the multiscale aligned microenvironment for neural outgrowth and extension within a 3D environment [175,176]. It remains an ongoing challenge to fabricate a multiscale composite scaffold that can provide a complex, biomimetic 3D structure for peripheral nerve regeneration. While previous studies have utilized hydrogels as bioinks to fabricate neural structures [177], these bioinks were generally unable to differentially regulate the cellular microenvironment required by different cell types to achieve physiologically relevant nerve regeneration. An additional challenge was to load growth factors such as neurotrophins within the printed scaffold, potentially with a spatially controlled concentration gradient, to encourage the formation of a 3D neural network.

Limited studies have explored 3D bioprinting of neural constructs using microgel-based bioinks. Chen et al. produced multiscale scaffolds using a granular microgel bioink, aiming to replicate the complex micro- and macro-environments found in native nerve tissue [177]. The bioink was made of the gelatin microgel and gelatin solution, comprising a discrete phase of microgels (enzymatically gelled gelatin microgels) and a cross-linkable continuous gelatin precursor solution-based phase containing transglutaminase (TG) precursor. As shown in Fig. 9A, GelMA and spherical chitosan microgels (GC-MSs) were fabricated using a microfluidic method and UV crosslinking (Step 1). The GC-MSs were then loaded with PC12 cells and nerve growth factor (NGF) to enhance neurite outgrowth and PC12 cell elongation (Step 2), followed by mixing with a GelMA hydrogel containing RSC96 Schwann cells to form a composite modular bioink (Step 3). This bioink was deposited layer-by-layer through extrusion bioprinting to form a neural scaffold (Fig. 9Ba). Cells encapsulated in this multiscale composite scaffold were found to have homogeneous distribution (Fig. 9B (b, c, e and f)), and the scaffold had smooth edges (Fig. 9B(d)). Additionally, the top view (Fig. 9Bg) and 3D view (Fig. 9Bh) were shown of GC-MS + NGF microgels seeded with PC12 cells (stained in green) in the GC-MS + NGF/GelMA scaffold encapsulated with RSC96 cells (stained in orange). The PC12 cells adhered and proliferated on the surface of GC-MS + NGF microgels, while RSC96 cells were homogeneously distributed in the modular bioink with some displaying elongated morphology, indicating that the GC-MS + NGF microgels promoted PC12 proliferation and neurite extension. Moreover, the strategy of bioprinting a RSC96 cell-laden hydrogel as a shell for encapsulating PC12 cell-laden GC-MS microgels can mimic the epineurium layer and drive nerve cell organization within a 3D environment. This study showed the versatility of employing a microgel-based bioink to print neural constructs and create a suitable spatially organized 3D environment for nerve tissue engineering.

Min et al. developed another approach for generating a peripheral nerve graft by 3D bioprinting, using decellularized extracellular matrix (dECM) and a reinforced PCL conduit [147]. The method involved an alginate microgel-based printing bath, which enabled the precise printing of a low-viscosity dECM hydrogel at 30 μm filament resolution and neutral pH. When applied to a rat sciatic nerve defect model, this bioprinted graft achieved comparable repair outcomes compared to the autologous nerve graft as a positive control, in the total number of regenerated axons and relative muscle weight ratio. This scaffold design held significant promise as an alternative to autologous nerve grafts for

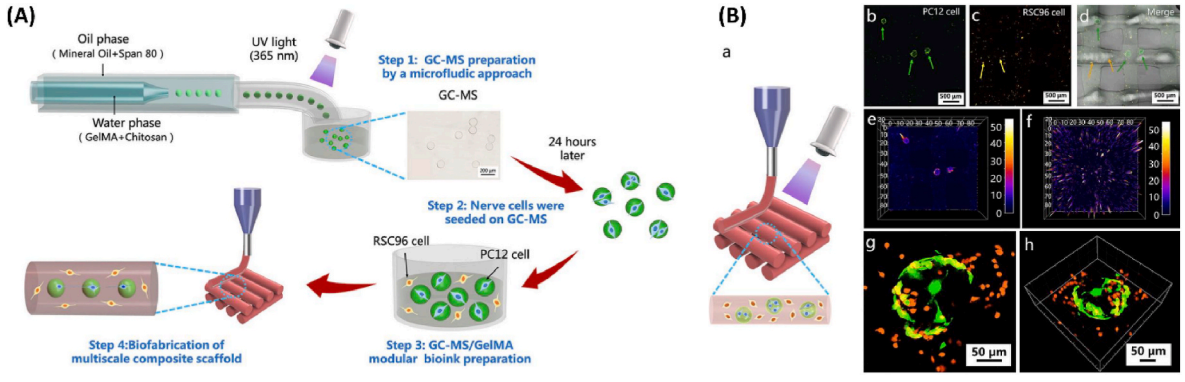


Fig. 9. Application of microgel-based bioink in nerve tissue engineering. (A) Fabrication of GelMA and spherical chitosan microgels (GC-MSs). (B) 3D bioprinting of the multiscale GC-MS + NGF/GelMA composite scaffold with PC12 cells and RSC96 cells. All images are reproduced with permission [177] under the terms of the Creative Commons Creative Commons CC-BY-NC-ND license. Copyright 2020, The Authors, published by Elsevier.

neural repair, benefitting from controlled microfilament printing enabled by the microgel printing bath.

8.2. Bone and cartilage tissue constructs printed with microgels bioinks

Despite significant advances in bone and cartilage tissue engineering, current research remains limited in the ability to repair critical-sized bone or cartilage defects [178,179]. Clinical treatments using autografts and allografts are limited by donor availability and high rates of graft resorption. Synthetic bone and osteochondral substitutes can offer mechanical support but typically lack bioactivity, while grafts made using natural polymers and their composites may create a suitable environment for tissue regeneration but typically lack sufficient mechanical strength for load-bearing applications. The balance among desirable scaffold properties, including biocompatibility, bioactivity, biodegradability, appropriate architecture, suitable mechanical properties for load-bearing, and the potential to integrate inductive growth factors, becomes increasingly challenging to achieve for critical-sized skeletal defects that potentially requires regeneration of interfacial tissues (such as osteochondral tissue) over a large surface area. 3D bioprinting can be useful in creating complex tissue constructs to meet these heterogeneous requirements [133,180]. Among bioink candidates for bone and cartilage tissue engineering, microgels provide the benefits of small size, injectability, and efficient encapsulation of live cells, making it possible to easily fabricate and deliver heterogeneous constructs to help with the regeneration of hierarchical or interfacial skeletal tissues [142,181,182].

Chai et al. introduced core-shell microgels with independently controlled compartments, designed to protect encapsulated cells during extrusion-based 3D bioprinting for bone regeneration [142]. The obtained microgels had a core comprising type I collagen and an outer shell containing alginate. These microgels were incorporated into a methacrylated silk fibroin (SilMA)/GelMA hydrogel to form a granular microgel-based bioink containing different concentrations of microgels. The bioinks were mixed with bone marrow mesenchymal stem cells for printing biomimetic bone constructs. Biocompatibility assessment indicated that the incorporation of microgels in the bioink exerted a protective effect on cells during printing and significantly enhanced cell proliferation, as the cell viability in the printed constructs printed with Microgel-15%SilMA/GelMA bioink was always higher than that in the printed constructs printed with 5%SilMA/GelMA, 10%SilMA/GelMA and 15%SilMA/GelMA conventional hydrogel-based bioinks. After the constructs printed with Microgel-15%SilMA/GelMA bioink and 15% SilMA/GelMA bioink were implanted subcutaneously in SD rats for 1 and 2 weeks, the two types of the constructs were surrounded by the soft tissues without showing obvious inflammatory response between the materials and the tissues (Fig. 10A). Therefore, the constructs printed with Microgel-15%SilMA/GelMA bioink had good biocompatibility and were suitable for *in vivo* implantation. After the cell-laden constructs were applied on skull defects of SD rats, the constructs printed from Microgel-15%SilMA/GelMA bioink showed better bone repair capability than the one printed from 15%SilMA/GelMA conventional bioink, indicating by the micro-CT results shown in Fig. 10B.

As discussed, in the earlier section, Flégeau et al. [108] fabricated tyramine-modified hyaluronic acid (HA-TYR) microgels bioink with human chondrocytes isolated from auricular cartilage mixed with the HA-TYR microgels creating three types of bioink with different microgel sizes and the traditional hydrogel-based bioink all supported cell viability. Interestingly, cell morphology was affected by the size of microgels, whereby the cells in bioinks containing microgels produced from 100 to 500 μm meshes rapidly formed small aggregates between the microgels. Conversely, cells in bioinks containing microgels produced from the 40 μm mesh were uniformly distributed and formed an interconnected network. The printed constructs supported the homogeneous development of mature cartilage-like tissues *in vitro* with progressive mechanical stiffening up to 200 kPa in elastic modulus after 63 days in culture, which also greatly exceeded the stiffness of constructs printed with conventional hydrogel-based bioinks. The mechanical strength of the printed construct could be reduced by increasing microgel size. Following 6 weeks of *in vivo* subcutaneous implantation in mice, the constructs formed from small diameter (40 μm) microgels remained stable with low immunogenicity and continuous tissue maturation. Interestingly, increasing the microgel size in the construct (100 and 500 μm) resulted in elevated inflammatory response and limited *in vivo* stability. The ability of the printed construct to induce cartilage regeneration in an *in vivo* osteochondral defect model was not tested.

9. Conclusion and perspectives

Though the field of tissue engineering has made tremendous progress in fabricating functional tissue substitutes based on scaffolding and microengineering, these substitutes are limited in their capacity to produce tissue constructs with precise biomimetic properties. 3D bioprinting offers a versatile platform to recreate the natural architecture of tissues and organs by delivering bioinks made of biomaterials and cells with precise control over their spatial distribution and architectural design. However, a significant challenge lies in formulating an ideal bioink that satisfies the multifaceted requirements for 3D bioprinting of diverse tissues and organs. Traditional hydrogel bioinks have been extensively researched, but their nanoporous structure restricts normal cell behavior, hampering endogenous cell infiltration and blood vessel integration post-implantation, often resulting in limited tissue regeneration and hence encountering challenges when considering clinical

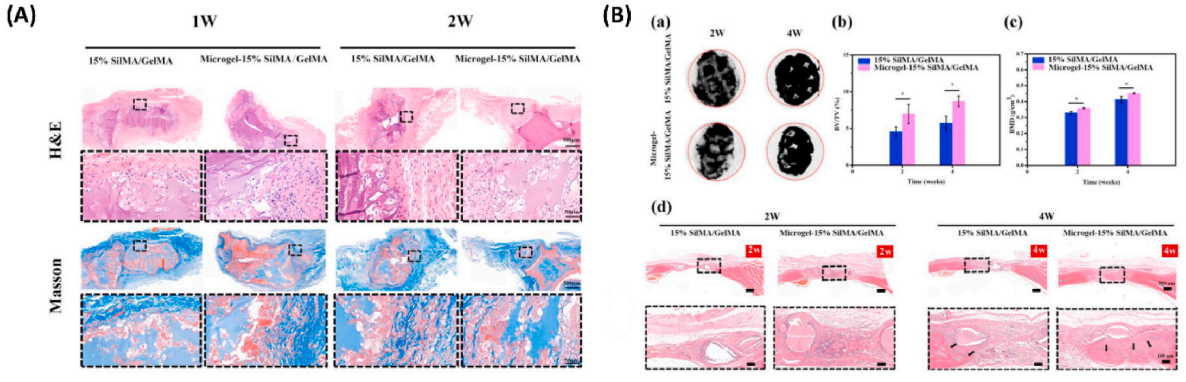


Fig. 10. Applications of microgel-based bioink in bone and cartilage tissue engineering. (A) H&E and Masson staining on the constructs printed with 15%SilMA/GelMA and Microgel-15%SilMA/GelMA bioinks after they were implanted for 1 and 2 weeks. (B) Bone repair in rat skull defects filled with 3D bioprinted 15%SilMA/GelMA and Microgel-15%SilMA/GelMA constructs. Reproduced with permission [142]. Copyright 2021, published by Elsevier Ltd.

applications. Hydrogel bioinks also face limitations in mechanical properties for 3D printing, lacking the requisite strength in their liquid state before crosslinking. This disrupts the balance needed between biocompatibility and printability, with current approaches often compromising one aspect over the other.

Microgels provide versatile capability to be tailored in properties such as shape, size, and responsiveness to external stimuli. Due to their ability to create micron-sized pores, microgels can offer protection to cells when they are being delivered in the bioink during 3D bioprinting, even under harsh printing conditions. Microgel bioinks inherently suit printing processes, enabling precise control over important parameters such as printability, porosity, and mechanical strength. The diverse array of microgel morphologies and materials opens the potential to simulate complex cellular microenvironments in bioprinting applications. In this review, we have comprehensively summarized the current state of research into microgel bioinks, which currently features a limited number of studies but is a dynamically growing area. We thoroughly discussed the microgel materials and synthesis methods, their physicochemical characteristics, techniques for assembling them into bioinks, printing modalities for producing bioprinted constructs, and potential applications in tissue engineering.

Despite the many advantages of microgel bioinks compared to bulk hydrogel inks for 3D bioprinting, the current research suggests a number of common limitations that need to be addressed for creating functional tissues and organoids, before commercial or more widespread applications can be considered. Although microgels have been intensively studied for drug and cell delivery, their use in fabricating granular hydrogel scaffolds through microgel assembly is just beginning to be explored. Many parameters relating to the printability of microgel bioinks and functionality of the resulting constructs warrant further investigation. Furthermore, the majority of current microgel bioinks contain microgels with spherical morphology or low aspect ratio, which may lead to low porosity and mechanical stability, and meanwhile cannot provide guidance to cells for anisotropic tissue formation. As discussed in our earlier review [58], high aspect ratio microgels may provide a better pore structure with greater pore size and porosity to enhance cell viability, proliferation, and migration. Limited investigations into synthesizing microgels with high aspect ratio calls for further research in this exciting area, which may inspire new types of microgel bioinks with broader functionality, such as for directing the alignment of cells in anisotropic tissues or to provide more stable mechanical properties.

As discussed in this review, several studies have directly compared bioinks made using microgels or bulk hydrogels comprising the same material(s), generally showing distinct advantages of microgel bioinks compared to their hydrogel counterparts. However, some limitations in these comparisons should be noted, firstly acknowledging the limited evidence available in this newly emerged area of research. Secondly, the comparisons are not comprehensive as the majority have focused on the pore structure of printed constructs, to emphasize the advantages of the microporous structure seen in constructs printed using microgel bioink. Other important aspects are rarely compared, such as bioink printability, and various properties of the printed constructs including their mechanical stability, mechanical strength, and effects of the constructs on cell behavior. Future research should focus on systematically comparing bioink printability and the properties of printed constructs, particularly their influence on cell behavior. The impact of microgel composition and structure on their mechanical properties at the molecular level should also be extensively studied. Specifically, the influence of factors such as polymer chain length, crosslinking degree, and polymer type warrants further investigation. Understanding whether these properties affect mechanical behavior through changes in intermolecular forces, chain entanglement, or other mechanisms could provide valuable insights for optimizing microgel performance. This will help establish a deeper understanding of the interactions between bioink characteristics and cellular responses, enabling the design of more effective bioprinted materials. Another factor that needs to be considered is the huge variations seen in the equipment and printing conditions employed in different studies. The type of 3D bioprinter used is inconsistent among studies, and each study tends to introduce a new set of customized printing parameters to produce optimal properties for the specific combination of printing materials or final application that the study has chosen to explore. A more systematic framework for producing and evaluating bioprinted constructs, particularly in practical applications of tissue regeneration, will help standardize experimental outcomes and enable more meaningful comparisons among studies.

Although microgel-based bioinks have shown some promise in bone and neural tissue engineering, these studies are still in their early stages and lack robustness. Additionally, the application of microgel-based bioinks in tissue engineering for other areas such as skin, lung, heart, ear, hand, and skull remain in its infancy, with limited and preliminary research. Advancing this field will require the development of microgel-based bioinks that can better replicate the physiological conditions and dynamic environments that cells experience within the body, thus improving the relevance and applicability of printed models for medical research and clinical applications. Moreover, efforts should focus on customizing bioink formulations and printing methods to meet the unique requirements of soft tissues, which could lead to significant breakthroughs in regenerative medicine and tissue repair for organs such as the liver, heart, and skin.

Current testing of microgel-based 3D bioprinted constructs in animal models is extremely limited, and experiments on large animals with injuries relevant to human pathophysiology are completely missing. For example, all current strategies for bioprinting bone and cartilage constructs using microgel bioinks have not applied the printed constructs in practically relevant models for skeletal tissue regeneration. The limited *in vivo* models used were either subcutaneous or non-load-bearing defects which are not truly representative of the efficacy of printed constructs in physiological bone or cartilage regeneration. Also, the potential for microgel-based printed constructs to revascularize and sustain ongoing cell survival and tissue repair once implanted into the body has not been explored. This is an area that could greatly benefit from microgel-based bioprinting, since constructs can be designed to have an inherent vascular network and specific patterns of cells to allow better tissue integration, which may be more easily realized using versatile microgel bioinks compared to bulk hydrogels.

The future trajectory of developments in 3D bioprinting is envisioned to prioritize research into microgel bioinks, focusing on fabricating microgels with various aspect ratios, shapes, and stiffness, and assessing bioink printability as well as the printed constructs in tissue regeneration and other applications. Specifically, the effects of different printing parameters and microgel properties on cell viability and downstream cell behavior need to be studied in detail, and the mechanisms for improved biological responses obtained using microgel-based bioprinted constructs in tissue regeneration need to be elucidated, which will prepare the field for translating microgel bioinks into more clinically relevant applications.

Declaration of competing interest

There are no conflicts of interest to declare.

CRediT authorship contribution statement

Keerthi Subramanian Iyer: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Lei Bao:** Writing – review & editing. **Jiali Zhai:** Writing – review & editing. **Aparna Jayachandran:** Writing – review & editing. **Rodney Luwor:** Writing – review & editing. **Jiao Jiao Li:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Haiyan Li:** Writing – review & editing, Writing – original draft, Supervision, Methodology,

Investigation, Formal analysis, Conceptualization.

Ethics approval and consent to participate

This is no need to obtain ethics approval and consent to participate for this review article.

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