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The microparticulate inks for bioprinting applications

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ABSTRACT

Three-dimensional (3D) bioprinting has emerged as a groundbreaking technology for fabricating intricate and functional tissue constructs. Central to this technology are the bioinks, which provide structural support and mimic the extracellular environment, which is crucial for cellular executive function. This review summarizes the latest developments in microparticulate inks for 3D bioprinting and presents their inherent challenges. We categorize micro-particulate materials, including polymeric microparticles, tissue-derived microparticles, and bioactive inorganic microparticles, and introduce the microparticle ink formulations, including granular microparticles inks consisting of densely packed microparticles and composite microparticle inks comprising microparticles and interstitial matrix. The formulations of these microparticle inks are also delved into highlighting their capabilities as modular entities in 3D bioprinting. Finally, existing challenges and prospective research trajectories for advancing the design of microparticle inks for bioprinting are discussed.

1. Introduction

Three-dimensional (3D) bioprinting boasts tremendous potential in generating tissue and organ mimetics, holding promise for personalized implants, tissue scaffolds, and in vitro diagnostic models [1]. This process necessitates the precise deposition of bioinks, amalgamations of biomaterials and/or cells, at specified 3D coordinates through computer-guided modalities [2]. Various bioprinting techniques, like inkjet, light-assisted, and extrusion-based methods, were recently reviewed, and it was emphasized that the design of bioinks should be tailored to each chosen technology [3–5]. Commonly, bioinks are crafted from various materials, including natural/synthetic polymers and polymeric composites [6], with many relying on polymer solutions that undergo gelation post-printing [7]. However, inkjet printing is suitable for small-sized structures, while shearing and thermal stress harm encapsulated cells. Light-assisted bioprinting has concerns about cell damage by UV exposure or photo-initiator [5]. It is considered that the polymer solutions flow before stabilization after extrusion printing, resulting in distortion of the printed structures. An alternative extrusion approach is pre-crosslinking polymer solutions to a higher viscosity state. Still, pre-crosslinking polymer networks have high shear stress during extrusion printing, reducing cell viability [8,9]. Besides, it is challenging to control the degree of crosslinking or gelation of the bioink to protect the embedded cells. Therefore, suitable inks should comply with several requirements: i) Appropriate rheological properties for optimal printability and shape fidelity [1]; ii) Biocompatibility and potential biodegradability that mimics in vivo conditions [10]; iii) Enhancement of biological activity to guide cell fate.

In recent years, researchers have started using various microparticulate ink formulations combined with printing technique-based strategies for generating modular building blocks to 3D print intricate bio-constructs and for realizing personalized implants [11–13]. The microparticulate biomaterials have developed, including naturally derived microparticles (Bioactive inorganic particles, particulate autologous tissues, and extracellular matrix (ECM) derived microparticles, etc.), polymeric microparticles (Microcapsules, microspheres, and

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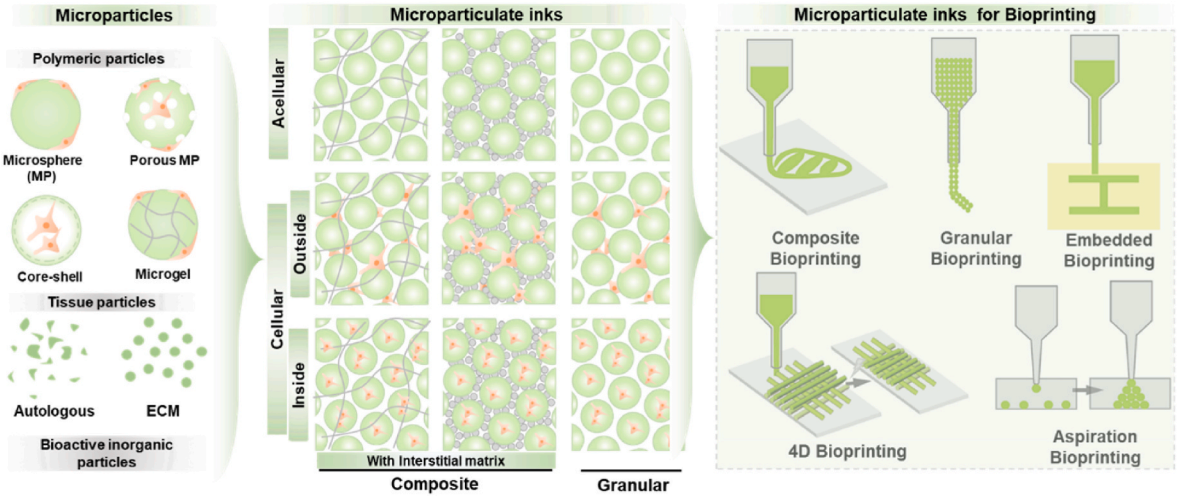


Fig. 1. Schematic representation of the microparticulate materials as inks used for bioprinting.

Table 1
The category of particulate biomaterials to generate different ink formulations.

Category	Particles type	Sources	Production method	Bioink type	Ref
Polymeric microparticles	Microspheres	Natural/Synthetic polymers	Batch emulsions/Microfluidic technique/ Photolithography/Electrostatic spraying	Composite/ granular MP inks	[20–22]
	Microgels	Natural/Synthetic polymers	Batch emulsions/Microfluidic technique/ Photolithography/Electrostatic spraying/Mechanical crushing	Composite/ granular MP inks	[23,24]
Tissue derived microparticles	Particulate tissues	Autologous/Allogeneic tissues	Grinding	Composite MP inks	[25,26]
	ECM particles	Decellularized/Demineralized tissues	Freeze-milling	Composite MP inks	[27–29]
Bioactive inorganic microparticles	Naturally derived particles	Diatomite	Natural compound	Composite MP inks	[30,31]
	Chemical synthetic particles	Calcium phosphate/Magnesium phosphates/Calcium peroxide	Chemical approaches	Composite MP inks	[32–34]

microgels, etc.), to generate different ink formulations to achieve bioprinting requirements [14,15]. For example, microparticles incorporated into polymers or colloids could improve printability and shape fidelity by adjusting rheological and mechanical properties and enhancing the biological performance of bioinks [16]. The microparticles act as culture platforms for cellular expansion or are used for cell encapsulation matrix, then these cellularized micro-forms as bioinks can engineer living constructs by bioprinting [15,17]. Additionally, microparticles could be densely packed with a jamming transition (Granular materials), where they behave like a solid until enough force is applied to induce movement [18]. The granular materials, which are dynamic structures with rheological properties of shear-thinning and self-healing, could be used for extrusion printing or to create suspension baths for embedded printing techniques [18,19]. With these excellent properties, microparticulate inks are important candidates for biological and biomedical applications such as cell/drug delivery, tissue engineering, sensing implants, etc. Therefore, the development and production of microparticulate ink may result in improved medical tools for advanced therapeutics.

This review emphasizes an overview of the recent advances in microparticulate materials for bioink formulation (Fig. 1). Firstly, we introduce the types and characteristics of microparticulate materials, including polymeric microparticles, tissue-derived microparticles, and bioactive inorganic microparticles. Subsequently, the microparticle ink formulations are detailed, including granular microparticle inks consisting of densely packed microparticles and composite microparticle inks comprising microparticles and interstitial matrix. Additionally, the formulations of these microparticle inks as modular entities in 3D bioprinting are discussed. Finally, a discussion on the current limitations, challenges, and prospects for advancing the design of microparticle inks is presented.

2. Classification of microparticles for generating various ink formulations

Over the past few decades, microparticulate biomaterials have gained increasing relevance in 3D bioprinting, and many different particulate formulations have been explored in the search for advancing bioinks [14]. We classify these formulations based on polymeric microparticles, tissue-derived microparticles, and bioactive inorganic microparticles (Table 1). When mixed with an interstitial matrix, microparticles enhance the printability and biofunctionality of 3D scaffolds, creating composite microparticle inks (MP inks), as described in this review. Granular MP inks refer to inks that consist of densely packed microparticles. Details of these categorizations are presented in the following sections.

2.1. The polymeric microparticles employed as inks for bioprinting

Recently, microparticles have increasingly been fabricated from polymers, which are ideal for 3D bioprinting due to their small size, unique physiochemical properties, excellent biocompatibility, and biodegradability [14,19,35]. The polymers include natural polymers (Collagen, gelatin, silk, hyaluronic acid, heparin, chitosan, dextran, cellulose, agarose, and alginate, etc.) and synthetic polymers (poly (lactic-*co*-glycolic acid) (PLGA), polycaprolactone (PCL), and poly

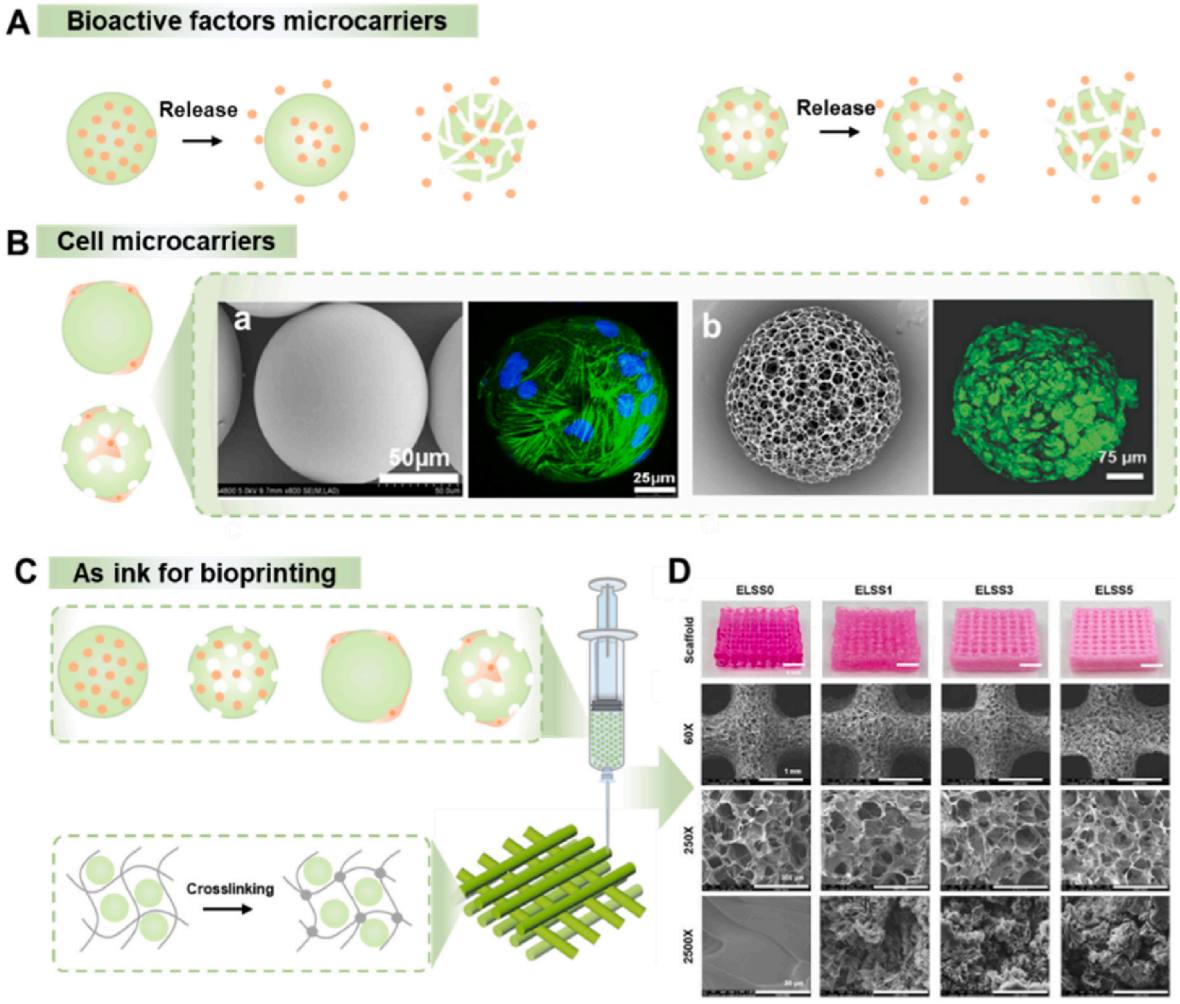


Fig. 2. The non-hydrogel based microparticles employed as inks for bioprinting. (A) Microspheres are used as bioactive factors microcarriers. (B) The SEM images of microspheres used as cell carriers. (a) The gelatin-based microcarrier. Reproduced with permission [45]. Copyright 2020, Elsevier. (b) The open-porous poly (lactic-*co*-glycolic acid) (PLGA) microspheres as cell carriers. Reproduced with permission [46]. Copyright 2019, Wiley-VCH GmbH. (C) The bioactive microspheres combined with an interstitial matrix for composite bioprinting. (D) The 3D printed stacked structure scaffolds. Reproduced under the terms of the CC BY 4.0 license [47]. Copyright 2023, the Authors. Published by Elsevier.

(lactic acid) (PLA), etc.). Polymeric microparticles (PMPs) with characteristic sizes of 1–1000 μm can be used as drug delivery reservoirs and well-explored as cell microcarriers [15,36]. Therefore, the PMPs as bioactive microspheres can be incorporated into polymer matrices to tune the biological performance of boinks. In particular, some polymers can be crosslinked to form water-swollen polymeric networks (Hydrogels), which hold large amounts of water or biological fluids [37]. Hydrogels with microscale particles are also called hydrogel microparticles or microgels. One part of hydrogel microparticles has a matrix core covered with a semipermeable membrane that is permeable to nutrients, oxygen, and metabolites and avoids the dispersion of the core content [36,38]. Thus, the core-shell microgels allow for recreating an appropriate cell microenvironment for developing living materials in which living cells are encapsulated into semipermeable membrane components [23]. The PMPs can be classified into non-hydrogel based (Microsphere) and hydrogel based (Microgel) microparticles (Table 1). These PMPs can be mixed with an interstitial matrix for composite bioprinting or jammed together for granular or embedded bioprinting, fabricating tissue-like constructs or organotypic structures. The PMP inks include composite and granular inks. Densely packed microparticles form the granular inks, while the composite inks comprise microparticles and interstitial matrix (Monomers, polymers, and nanoparticles). The PMP inks combined with cells generate cellular inks in which the cells are seeded on the PMP surfaces (Outside) or encapsulated into PMP units (Inside). In addition, the PMPs can be used to advance printing approaches such as 4D bioprinting.

2.1.1. The non-hydrogel based microparticles employed as inks for bioprinting

The non-hydrogel based microparticles (Microspheres) have emerged as promising vehicles for the delivery of cargo (i.e., drugs, bioactive factors, and cells) (Fig. 2A and B) [39]. Microspheres are defined as tiny spherical particles, ranging in size from 1 to 1000 μm, mainly produced through a simple method based upon forming spheres [40,41]. The fabrication processes were divided into chemical methods (i.e., interfacial polymerization and coacervation) and physical methods (i.e., atomization and extrusion techniques. For a more detailed comparison, please refer to review by Itawi et al. [42]. Here, according to the surface properties, microspheres are divided into solid and porous microparticles. In the case of a polymer matrix, the diffusion of cargo (i.e., drugs and bioactive factors) can be through the polymer network or the pores of porous microspheres (Fig. 2A). When the polymers are biodegradable, the cargo can be released by breaking down the microspheres [43]. Microspheres' controlled porosity and pore structure offer the smart properties of releasing the loads (i.e., drugs and bioactive factors) with pathological response switches for treating diseases [44]. The

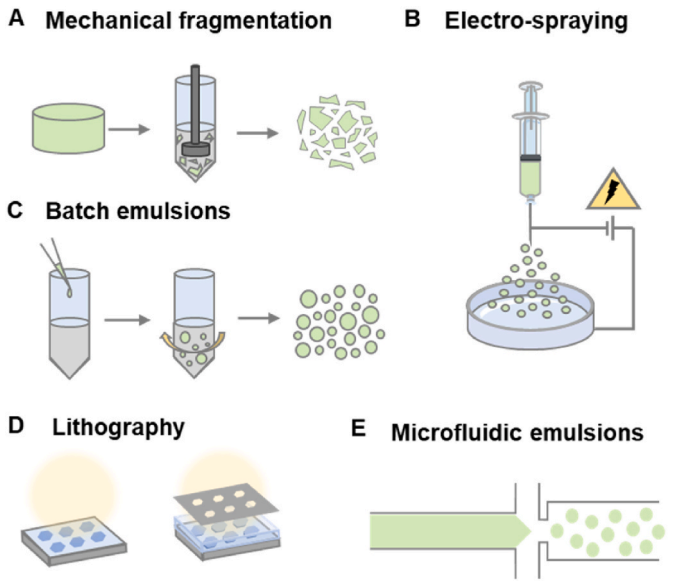


Fig. 3. Overview of the microfabrication techniques of microgels. (A) Mechanical fragmentation, where bulk matters are fragmented into microparticles using homogenizer devices. Adapted from Widener et al. [56]. (B) Electro-spraying, where precursor solution is electro-sprayed in an oil dish for the rapid and scalable formation of droplets that are then jellified to form microgels. Adapted from Qazi et al. [57]. (C) Batch emulsions, where precursor solution is mixed with an immiscible fluid (e.g., oil) to form droplets via emulsification. Adapted from Widener et al. [56]. (D) Lithography, where masks or moulds are used as templates for microgel fabrication. Adapted from Daly et al. [35]. (E) Microfluidic emulsions, where the uniform emulsion droplets are generated through a microfluidic chip, then precursor solution in these droplets crosslinked to produce microgels. Adapted from Qazi et al. [57].

microspheres can also be used as cell microcarriers for the *ex vivo* expansion of adherent cells or as building blocks for modular tissue engineering and regeneration (Fig. 2B) [15,45]. The cells can increase on the surface of solid microspheres, while porous microspheres facilitate the cells to migrate in the pores and provide more spaces for cell growth [46]. More recent trends have explored the possibility of using microsphere incorporation to fine-tune the biological and mechanical performance of bioinks (Fig. 2C and D). The microspheres and various types of interstitial materials can extrude as stable filaments in which the interstitial matrix can be further crosslinked to form stable structures (Fig. 2C). Seok and colleagues presented a 3D printed bioactive microparticle-loaded scaffold for bone regeneration applications (Fig. 2D) [47]. Dixon and colleagues exhibited the efficient encapsulation and release of bioactive factors (osteogenic transducing transcription factor RUNX2 protein) from PLGA microspheres, which could be combined in a mechanically strong and printable material [48]. In addition, Mateos-Timoneda and colleagues developed cell-laden polylactic acid (PLA) microcarriers combined with gelatin methacrylamide-gellan gum bioinks for fabricating living osteochondral constructs via 3D bioprinting [49]. It was demonstrated that PLA microcarriers acted as reinforcement units to increase the mechanical strength of structures and offered cell-colonized sites to support osteogenic differentiation. Generally, bioactive cargos or cells-laden microspheres incorporating printable matrices can fabricate biofunctional structures or tissue-like constructs. Despite progress, challenges like nozzle clogging and potential toxic byproducts remain unresolved [15].

2.1.2. Hydrogel based microparticles (Microgels) employed as inks for bioprinting

2.1.2.1. The microfabrication techniques of microgels. The microgels can be fabricated using various microfabrication techniques, including mechanical fragmentation, electro-spraying, batch emulsions, and lithography (Fig. 3) [17]. We refer the reader to other remarkable reviews for a more detailed overview of microfabrication techniques [35, 50–53]. These microgels can be densely packed to form granular hydrogels, which exhibit the shear-thinning behavior of 'solid-like' at low strain and 'fluid-like' at high strain, and these properties are ideal for extrusion bioprinting [20,21]. Jammed microgels have also been employed as supporting baths for 3D printing structures (Embedded bioprinting) made from diverse soft matter components [19,54]. In addition, Anseth and colleagues have given the idea that microgels uniquely provide highly tunable microenvironments for manipulating cell fate, as well as a building block to recapitulate tissue mimics [17]. Therefore, these cell-laden microgels have been explored as inks to print complex tissues [20,55]. More in-depth discussions of granular gel inks are presented below.

2.1.2.2. The microgel inks. During extrusion bioprinting, ink printability generally refers to the "suitable" extrudability, filament formation, and shape fidelity [58]. The rheological properties of bioinks are often used as predictors of printability and shape fidelity. Compared to hard-spherical particles, soft microgels have complicated rheological properties due to the physicochemical properties of microgels, such as shape, concentration, interparticle interactions, composition, size, and stiffness (Fig. 4A) [20]. When the low concentration of microgels is suspended in the continuous phase, the particle-to-volume fraction Φ is tiny. With increasing Φ, the microgels become concentrated, and the physical properties of jamming microgels behave like a solid. Compared to colloids, these jamming microgels with relatively larger particle sizes have unique properties, such as interparticle interactions controlled by gravitational forces rather than thermal forces (Fig. 4B(a)) [59]. The inter-microgels are predominantly influenced by friction more than attractive van der Waals forces. When the particle-to-volume fraction is above $\Phi \approx 0.58$, the packed microgels undergo a jamming transition called random loose packing. While closer to $\Phi \approx 0.64$ (Defined as random close packing), microgels can be treated as a solid with all properties of conventional hydrogels. Significantly, the Φ value of jammed soft microgels (i.e., gelatin microgels [60]) is higher than 0.64, as microgels are deformable and irregular after being processed using packing techniques [20]. Whatever the Φ value of jammed microgels is, particles can displace when sufficient stress is applied (yield stress) and quickly self-heal into a whole bulk when the applied stress is removed (Self-healing) [21,60,61]. In addition, viscosity contributes to shape fidelity, where low viscosity can readily flow within the needle, and high viscosity maintains shape and structural stability after extrusion [62]. A decrease of viscosity with increasing shear rate (Shear-thinning) is a fundamental rheological property describing printability and printing fidelity (Fig. 4B(b)). The jammed microgels at high packing densities have been proven to exhibit shear-thinning behavior [63]. Therefore, the unique rheological properties of jammed microgels, including yield stress behavior, shear-thinning, and self-healing, exactly meet the rheological requirements for extrusion-based 3D bioprinting, inspiring their use as bioinks (Fig. 4C(a)) [64].

In addition, the physicochemical properties of microgel inks also affect the printing stability, and shape fidelity. For example, Flégeau et al. demonstrated that printing precision was directly correlated to microgel size and unrelated to wider pathwidth [66]. As the microgel size increases, the printing resolution of the structure decreases sharply, and smaller size microgels have better resolution. The microgel with the appropriate diameter can ensure the extrusion of smooth continuous filaments and prevent nozzle clogging [21]. The effects of microgel inks' mechanical properties on shape fidelity were explored, and increasing structural stability with increased microgel modulus was observed in hollow cylinder printed structures [21,67]. Notably, stiffer microgels may be detrimental to cell survival due to the high shear stress during the printing process. For softer microgels, additional conditions may

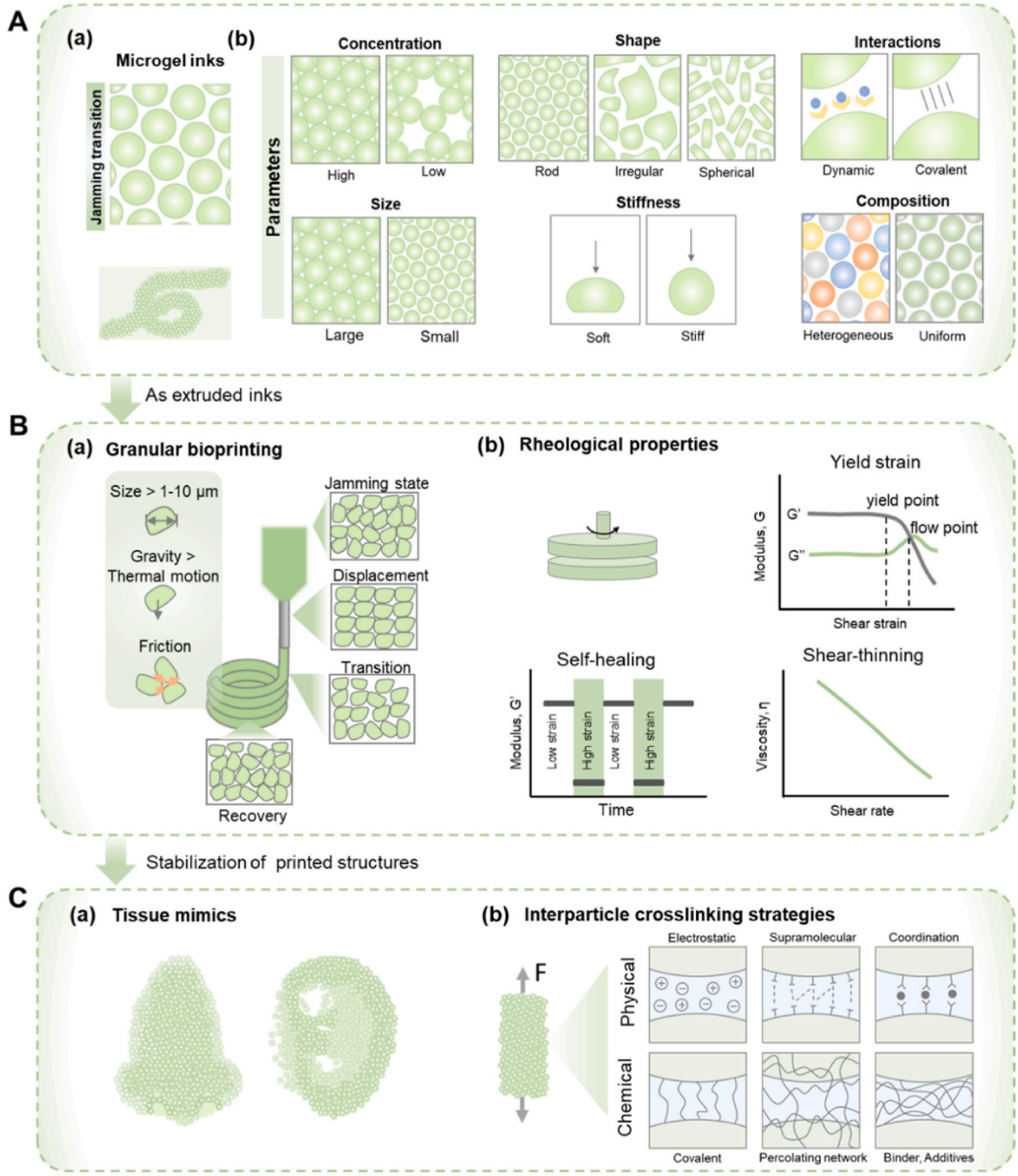


Fig. 4. Schematic representation of the jammed microgel inks for 3D printing applications. (A) The effect of physicochemical parameters of microgel on microgel ink printability. (a) The jamming transition of microgel ink with (b) the physicochemical factors, such as shape, concentration, interparticle interactions, composition, size, and stiffness, adapted from Daly et al. [20]. (B) Overview of jammed microgel as extruded ink for granular printing. (a) Properties for microgel and transition of jammed microgel ink during the extrusion printing process. (b) The rheological properties necessary for printability of jammed microgel ink, such as yield strain, self-healing, and shear-thinning. (C) Strategies of stabilization of printed structures after printing. (a) Schematic of nose-shaped and ear-shaped structures printed using jammed microgels. (b) Interparticle crosslinking enhances printed designs' stability, adapted from Charlet et al. [65].

need to be considered to improve the shape fidelity of the printed constructs. Together, these results indicate that precision engineering microgel properties should balance cytocompatibility, printing stability, and shape fidelity for functional outcomes during bioprinting.

2.1.2.3. The composite microgel inks. Despite the ease of achieving self-healing after printing jammed microgels, the printed structures still require enhancement of mechanical stability for different application scenarios due to the weak physical and frictional interactions between microgels (Fig. 4C(b)). To improve mechanical stability of the printed structures, the interparticle interactions of jammed microgels can be modulated by the introduction of interparticle chemical crosslinking between microgels (Fig. 4C(b)) [68–70] or by using embedding interstitial matrices to offer second network (Fig. 5A, B and C) [67,71,72], or introducing nanoparticles to change the interfacial properties of adjacent microgels (Fig. 5D and E) [73,74]. The strategies of interparticle crosslinking between microgels, including covalent crosslinking and dynamic crosslinking, have been summarized in other notable reviews

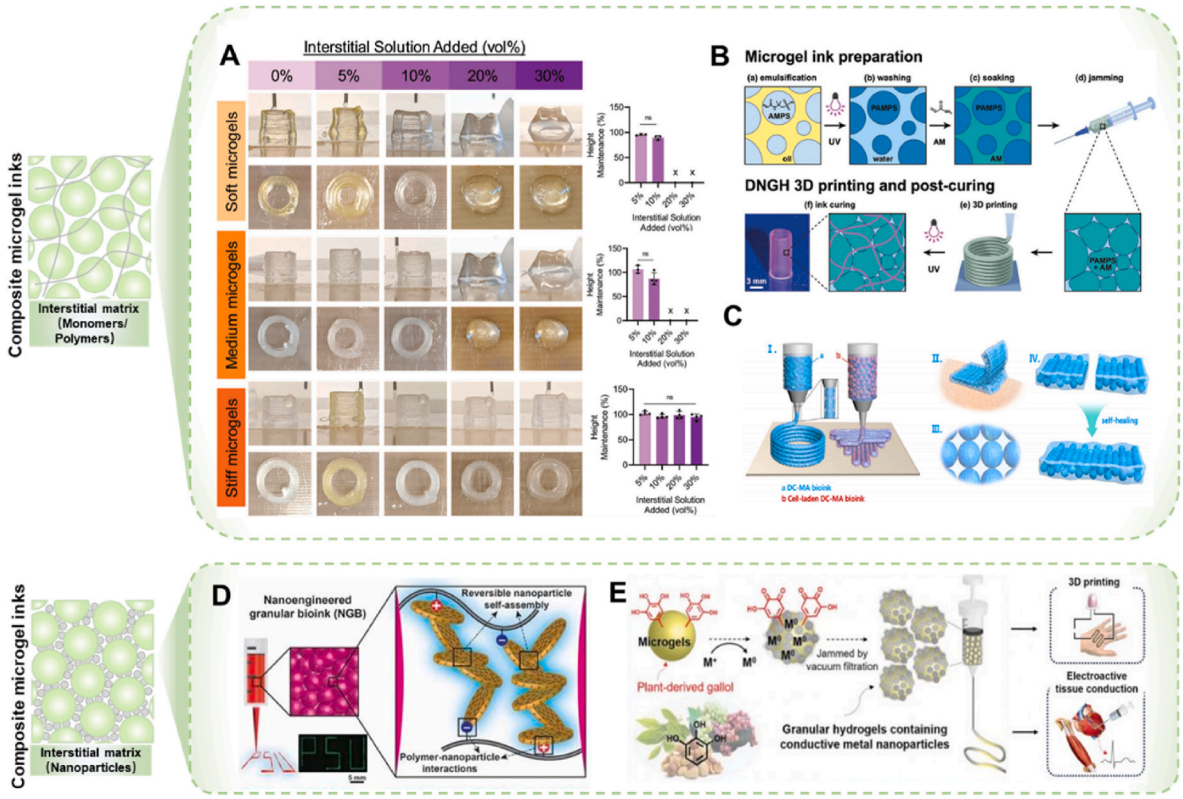


Fig. 5. The effect of composite microgel inks on mechanical response and additional functionalities of the printed structures. (A) Extrusion printing with microgels and interstitial matrix compositions in which microgels of varying compressive moduli (Soft, medium, stiff, 10–70 kPa) are combined with interstitial matrices (0–30 vol%) with compressive moduli ranging from 2 to 120 kPa. Reproduced under the terms of the CC BY 4.0 license [67]. Copyright 2023, the Authors. Published by Wiley-VCH GmbH. (B) Microgels are soaked in an acrylamide (AM) monomer solution, where these monomers are converted into a percolating network through exposure to UV light, resulting in strong and tough soft materials. Reproduced with permission [71]. Copyright 2022, Wiley-VCH GmbH. (C) Microgels incorporated into a dynamic cross-linker, dopamine-modified hyaluronic acid (HA-DA), formulate a dynamic bioink. Reproduced with permission [72]. Copyright 2022, American Chemical Society. (D) The nanoengineered granular bioink (NGB) combines GelMA microgels with heterogeneously charged silicate nanoparticles to yield shear-thinning bioink. Reproduced with permission [73]. Copyright 2022, Wiley-VCH GmbH. (E) The conductive granular ink, where the hyaluronic acid (HA) microgels containing in situ synthesized silver nanoparticles (AgNPs) are jammed into granular inks with conductive properties for 3D printing. Reproduced under the terms of the CC BY 4.0 license [74]. Copyright 2019, the Authors. Published by Wiley-VCH GmbH.

[35,65,75]. While interparticle crosslinking results in more stable and rigid structures, the stretchability of the jammed microgel materials becomes weak, reducing their toughness [71]. Researchers introduce a composite approach in which a cross-linkable interstitial matrix resides between microgels, allowing microgel ink flow during printing and reinforcing the stability of printed structure via crosslinking after printing. Here, the inks composed of jammed microgels and the interstitial matrices are called composite microgel inks. Burdick and coworkers investigated that varying the compressive modulus of microgels (10–70 kPa) and interstitial matrices (2–120 kPa), with interstitial volume fraction (0–30 %), influences the jammed microgel properties and it applied to 3D printing (Fig. 5A) [67]. The increased microgel modulus confers high structural stability in the composite microgel inks. Amstad and colleagues introduced an ink composed of swollen microgels in a monomer-containing solution, and these monomers were converted into a percolating network after printing, resulting in a solid and rigid double network (Fig. 5B) [71]. Dong and colleagues developed a dynamic bioink in which microgels are incorporated into a dynamic cross-linker, dopamine-modified hyaluronic acid (HA-DA) (Fig. 5C) [72]. The interstitial matrix (HA-DA) expanded the function of the resulting manufacture. Recently, researchers have developed inter-microgel interactions via the interfacial self-assembly of nanoparticles, which can preserve the microscale pores among microgel inks during and after bioprinting. For example, Sheikhi and colleagues developed a class of granular bioinks in which heterogeneously charged silicate nanoparticles readily adsorb onto GelMA microgels and reversibly self-assemble at the interface, yielding shear-thinning nanoengineered granular bioinks with well-preserved microscale porosity for the 3D extrusion bioprinting of tissue engineering scaffolds (Fig. 5D) [73]. Burdick and colleagues introduced a new concept in developing injectable and conductive jammed hyaluronan (HA) microgels with metal-phenolic coordination networks [74]. The HA polymer chain is modified with gallol moieties capable of oxidizing to form galloquinones and donate electrons. Metal ions (e.g., M^{+}) are coupled with this gallol oxidation, generating metal nanoparticles (e.g., M^{0}) that form coordinated networks with gallol groups. Therefore, this in situ synthesis of conductive granular gel is an attractive approach to improve conductivity and mechanical properties due to metal nanoparticles at the jammed interface with high surface area in this unique design. The conductivity of the nanoparticle-microgel ink can be applied to the 3D printing of numerous applications where conductive materials are needed (Fig. 5E).

2.1.2.4. The microgel inks with living components. Engineered living materials have emerged from combining living cells with advances in complex materials, towards increased applications from cell delivery for regenerative medicine to microbial bioprocessing to produce high-value chemicals [76,77]. Bioprinting enables the programmable deposition of bioinks containing living cells to recapitulate the intricacy and biofunctionality of native tissues [78–80] or to enhance bioprocessing [81,82]. This section discusses bioprinted living structures that combine microgel inks with living components such as mammalian cells and

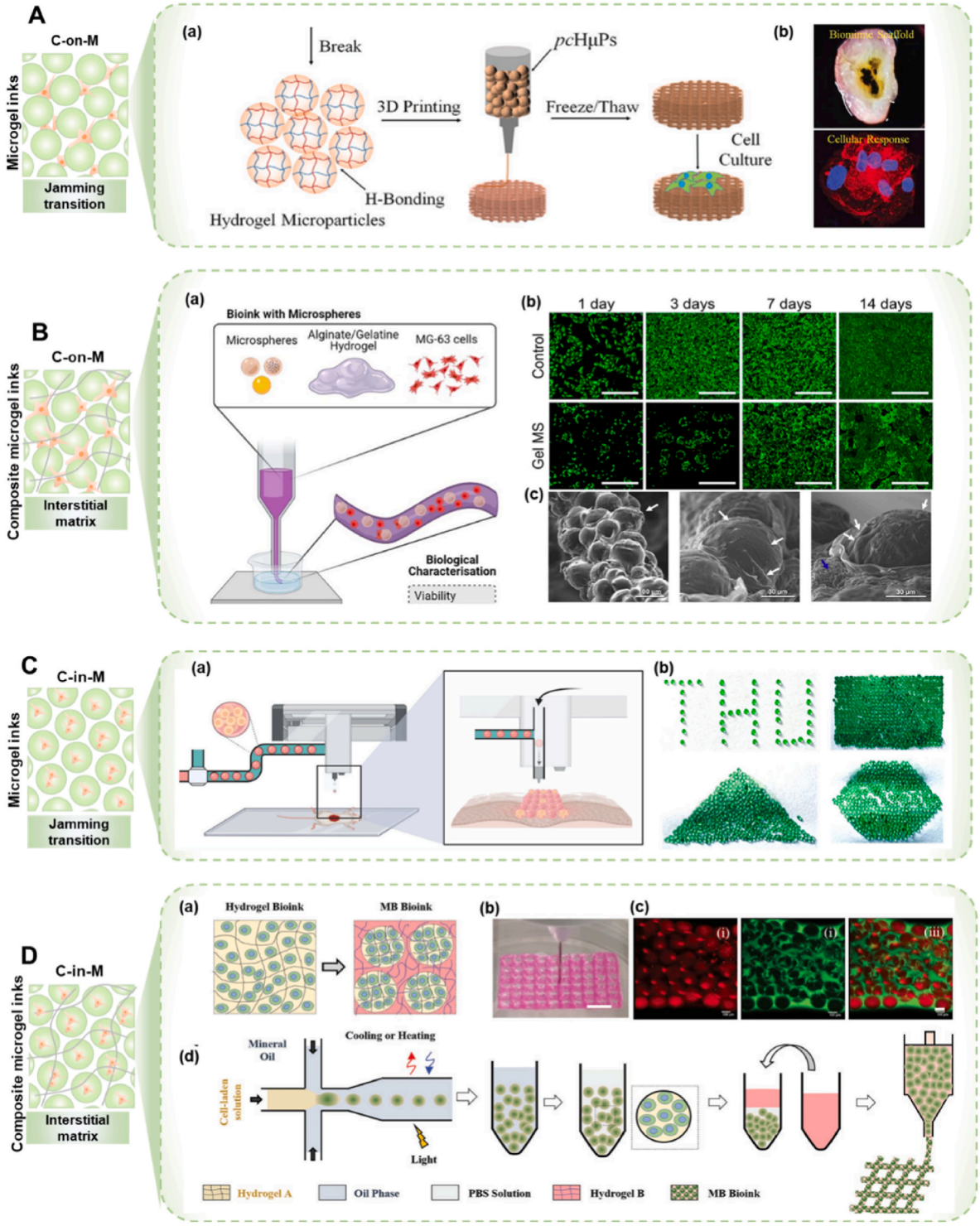


Fig. 6. The microgel inks are combined with mammalian cells for bioprinting. (A) 3D Printed biomimetic scaffolds based on jammed microgels mixed with cells. (a) The fragmented hydrogel into microparticles form jammed states through hydrogen bonding, which is used as inks for the 3D printing of biomimetic structures (A human-size ear model) and scaffolds to support cell adhesion and growth (b). Reproduced with permission [86]. Copyright 2020, Wiley-VCH GmbH. (B) 3D printing of composite bioink formulation containing a hydrogel with cell-adhering microspheres and cells. (a) The combining an alginate/gelatin hydrogel with cell-adhering microspheres and cells as bioinks. (b) Live/dead staining images on days 1, 3, 7, and 14. Live cells are stained in green and dead cells in red. Scalebar = 500 μm. (c) SEM images of the bioink where arrows indicate cells attached and spread on the MS. Reproduced under the terms of the CC BY 4.0 license [87]. Copyright 2022, the Authors. Published by Sage Publications. (C) Printing of MSCs laden matrigel beads augmenting skeletal muscle and hair follicle regeneration. (a) High throughput intra-operative formulation and printing of MSCs-laden Matrigel beads. (b) The homogenous structures of printed gelatin beads include a THU alphabetical sparse pattern and rectangular, triangular, and hexagonal dense patterns. Scale bar = 5 mm. Reproduced under the terms of the CC BY 4.0 license [88]. Copyright 2022, the Authors. Published by Springer Nature. (D) 3D printing of cell-laden microgel-based biphasic bioink. (a) Schematic representation of microgel combined with hydrogel to form biphasic ink. (b) 3D printing grid structure with biphasic ink. (c) The biphasic bioink consists of GelMA microgels (Red fluorescence) as a discrete phase and a second 5.0 wt% GelMA polymer network (Green fluorescence) as a continuous phase. (d) Step-by-step illustration of the fabrication process of biphasic bioink. Reproduced with permission [89]. Copyright 2021, Wiley-VCH GmbH.

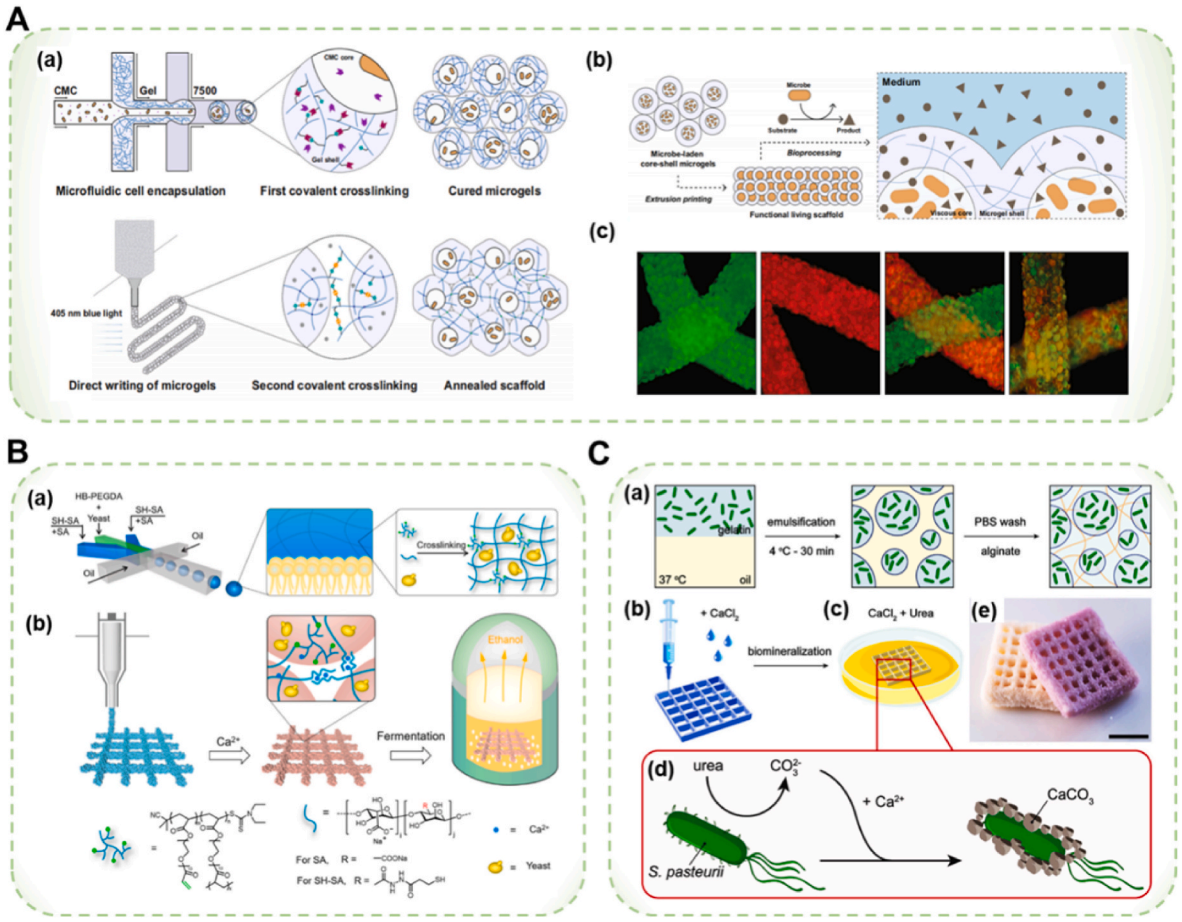


Fig. 7. The microgel inks are combined with microorganisms for bioprinting. (A) Microfluidic templating of core-shell microgel ink for fabricating scaffolds. (a) The schematic chart shows that the microbe-laden core-shell microgels are generated via droplet microfluidics and jammed together for extrusion printing. (b) The printed scaffolds are used for bioprocessing, where the microorganisms act as whole-cell biocatalysts to convert the substrates into products in the medium. (c) Fluorescent images of extruded filaments. Reproduced under the terms of the CC BY 4.0 license [23]. Copyright 2023, the Authors. Published by Springer Nature. (B) Schematic of 3D printing of biocatalytic living materials using microgel inks. (a) The fabrication of yeast-laden microgels via droplets microfluidics and crosslinked by Michael addition reaction. (b) The 3D printing of biocatalytic scaffolds was solidified upon adding Ca^{2+} and then used for fermentation. Reproduced with permission [92]. Copyright 2022, Elsevier. (C) Fabrication of 3D printed biomineral composites. (a) Schematic representation of bacteria-loaded microgel ink combined with an alginate solution before jamming. (b) A Ca^{2+}-crosslinked alginate network stabilizes the 3D printed structure. (c) The scaffolds were immersed in a composite solution containing $CaCl_2$, urea, and yeast. (d) Schematic illustration of the microbially-induced calcium carbonate precipitation (MICP) process mediated by S. pasteurii. (e) Photograph of a 3D printed biomineral composite after 4 days of MICP. Scale bar = 10 mm. Reproduced under the terms of the CC BY 4.0 license [12]. Copyright 2023, the Authors. Published by Elsevier.

microorganisms.

The microgels have been reported either as mammalian cell culturing microcarriers (i.e., cells are seeded on the microgel surfaces, C-on-M) or to recapitulate 3D microenvironments (i.e., cells encapsulated within microgel units, C-in-M) [17,83,84]. These cellularized microgels can be used for the bottom-up engineering of complex 3D constructs due to their modular and customizable characteristics [85]. Firstly, we introduce the different formulations of cellularized microgel inks for 3D bioprinting, including C-on-M/C-in-M microgel inks or their composite formulations (Fig. 6). For example, Fu and colleagues developed self-healable pre-crosslinked hydrogel microparticles of chitosan methacrylate used as C-on-M microgel inks, which favors a steady and continuous printing for a series of biomimetic constructs (Fig. 6A) [86]. These 3D printed scaffolds modulate the merging of bone marrow-derived mesenchymal stem cells (MSCs) into spheroids and help maintain the stemness of MSCs, which are significant for tissue regeneration. To improve the mechanical and tune the biological performance of bioinks, microgels incorporated into the matrix are used as bioinks. Ginebra and coworkers incorporated gelatin microspheres into alginate/gelatin hydrogel to form composite microgel inks (Fig. 6B) [87]. The results demonstrated that gelatin microspheres incorporated in the hydrogel improved the rheology and shape fidelity of the inks, supported cell viability and migration, and further positively affected the osteogenic differentiation of cells.

Beyond serving as C-on-M platforms to support cellular expansion, microgels can be used as C-in-M platforms where they can be tailored to precisely control the cellular microenvironment for promoting cell survival, promoting cell-matrix interactions, and guiding cell fate decisions [17]. Therefore, compared to C-on-M microgel inks, cells can also be encapsulated directly within microgels to generate C-in-M microgel inks. Ma and colleagues designed a “bead-jet” printer that integrated high-throughput generating and printing of MSCs-laden Matrigel beads (Fig. 6C) [88]. The results demonstrated that high-density encapsulation of MSCs in Matrigel beads can enhance the bio-functionality of MSCs, such as increasing cell proliferation, migration, and secretion, compared with low-density bead or high-density bulk encapsulation of an equal number of cells. In vivo, the high-density MSCs-laden beads printed with sparse patterns significantly improved therapeutic performance. However, microgel inks often lack durability depending solely on microgel physical interactions. Addressing this, Xiong et al. introduced a dual-phase bioink combining GelMA microgels and a GelMA hydrogel precursor (Fig. 6D) [89]. The jammed microgels improve rheological

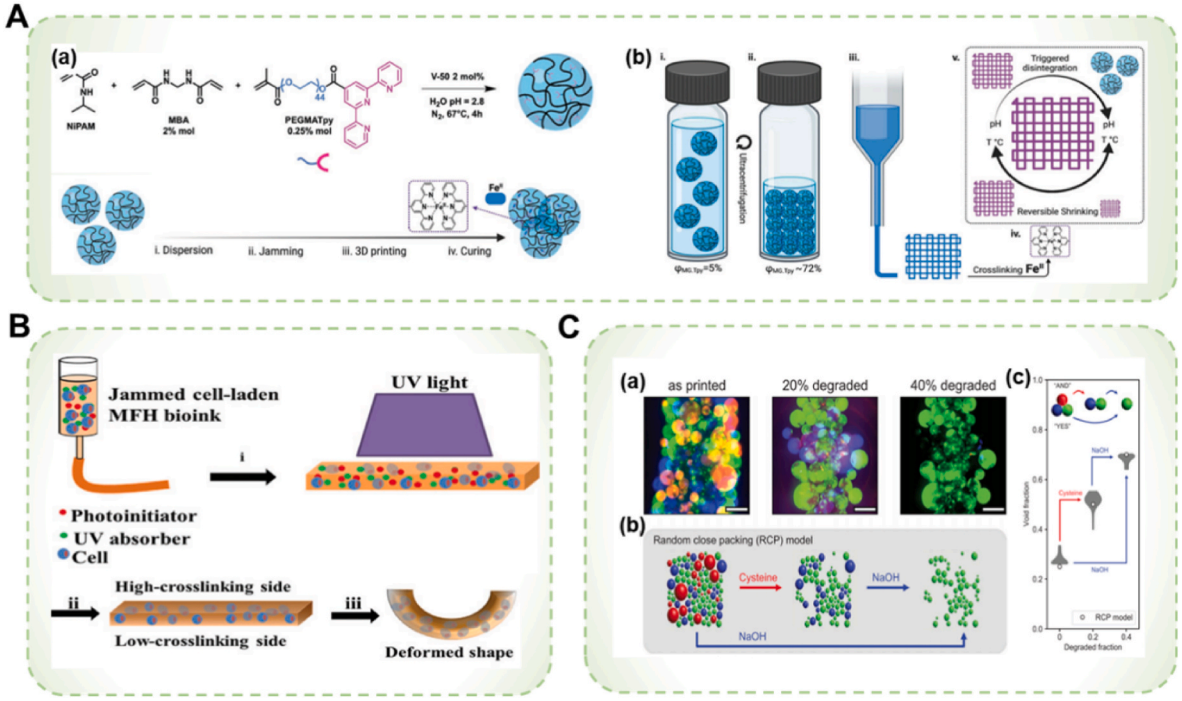


Fig. 8. The microgel inks with smart stimuli-responsive materials for printing. (A) A temperature and pH stimuli-responsive microgel ink. (a) Synthesis of terpyridine-modified thermoresponsive poly (N-isopropylacrylamide) (pNiPAM) microgels. (b) The 3D printed structures with pNiPAM microgel ink have properties of either cyclically shrunk-expanded by variation of the temperature or dissolved by adjusting the pH. Reproduced under the terms of the CC BY 4.0 license [13]. Copyright 2022, the Authors. Published by Wiley-VCH GmbH. (B) Jammed microgel for 4D living cell bioprinting. The crosslinking gradient of the printed 3D constructs allowed for shape morphing after being cultured in cell culture media. Reproduced with permission [96]. Copyright 2022, Wiley-VCH GmbH. (C) Logic-gated degradation to achieve scaffold void fraction. (a) Void fractions of printed microgel filaments were tuned by degrading thioester (Red) and ester (Blue) microgels while retaining amide (Green) microgels. Scale bars = 500 μm. (b) A random close packing (RCP) model of jammed microgels was used to analyze logic-gated changes in void space. (c) The experimental data of scaffold void fraction agreed with the RCP model. Reproduced with permission [98]. Copyright 2022, Wiley-VCH GmbH.

properties for extrusion bioprinting, while the precursor can integrate the microgels and enhance post-printing structural stability by forming a second polymer network.

Apart from mammalian cells, microgels can be combined with microorganisms to create functional bioprinted structures. Knowles et al. explored cell-laden core-shell microgels for bioprinting and creating functional living materials (Fig. 7A) [23]. The core-shell microgels were fabricated via droplet microfluidics, where microorganisms were encapsulated in the carboxymethylcellulose (CMC) core phase, and the transglutaminases were diffused from the CMC core into the gelatin/gelatin methacryloyl (GelMA) shell phase to catalyze the formation of the first covalent crosslinking in microgel shells (Fig. 7A(a)). Here, the CMC core and GelMA shell phases were formed by phase separation above critical concentrations, called an aqueous two-phase system [90, 91]. After extrusion printing, the bacteria-loaded core-shell microgels can proceed with a second covalent crosslinking among particles. Double bonds in GelMA can trigger crosslinking by 405 nm blue light. Then, the core-shell microgel ink was used to print functional living scaffolds for bioprocessing (Fig. 7A(b)). The results demonstrated that this scaffold supported the growth of microbial populations and reduced the cell leakage to the medium while remarkably improving the bioactivities of microorganisms. In addition, the microgel ink could pattern into various structures, including homogenous and heterogeneous structures (Fig. 7A (c)). Yu and coworkers developed biocatalytic living materials by encapsulating microorganisms within microgel inks for 3D printing (Fig. 7B) [92]. The results showed that microgels can protect yeast cells from shear forces during 3D printing and enhance the catalytic activity of yeast cells. Amstad and colleagues combined gelatin microgel inks with biopolymer alginate to fabricate bacteria-induced biomineral composites (Fig. 7C) [12]. The gelatin microgels were used to encapsulate S. pasteurii and then centrifugated to form a jammed state as ink (Fig. 7C(a)). The alginate could gel in the presence of Ca^{2+} and also serves as a Ca^{2+} reservoir combining CO_3^{2-} induction by S. pasteurii to facilitate situ growth of $CaCO_3$ (Fig. 7C(b-d)). Finally, the $CaCO_3$ minerals are accumulated within the 3D printed scaffold, transforming into a biomineral composite (Fig. 7C(e)).

2.1.2.5. The microgel inks for personalized implant printing. The increasing efforts have focused on developing microgel inks to obtain high printability and fidelity of the bio-constructs. The microgel inks with extended functions have recently been proposed and developed for realizing personalized implants. A precision implant uses customized material chemistry, bioactive components, and/or pathological features to detect or treat disease or injury in patients [93]. Through reasonable design and control of physical and chemical properties of microgels, the use of microgels as unit operators can enable the generation of personalized implants for a specific diseased environment within a patient [94]. For example, the microgel ink has a feedback mechanism where degradation, deformation or swelling occurs in response to local changes in temperature, pH, or biochemical molecules, resulting in therapeutic actions. Recently, Włodarczyk-Biegun and colleagues developed a new microgel ink with reversible temperature-induced resolution enhancement and on-demand disintegration (Fig. 8A) [13]. The microgel ink was generated from jammed sub-micrometer poly (N-isopropylacrylamide) (pNiPAM) microgels containing terpyridine (Tpy) ligands for the formation of reversible inter-particle crosslinking (Fig. 8A(a)). Because the Tpy moieties can form metal-ligand bonds from neighboring particles, the printed scaffold can be instantly fixed when immersed in an iron (II) ions solution (Fig. 8A(b)). Due to the thermosensitive pNiPAM with Lower Critical Solution Temperature (LCST,

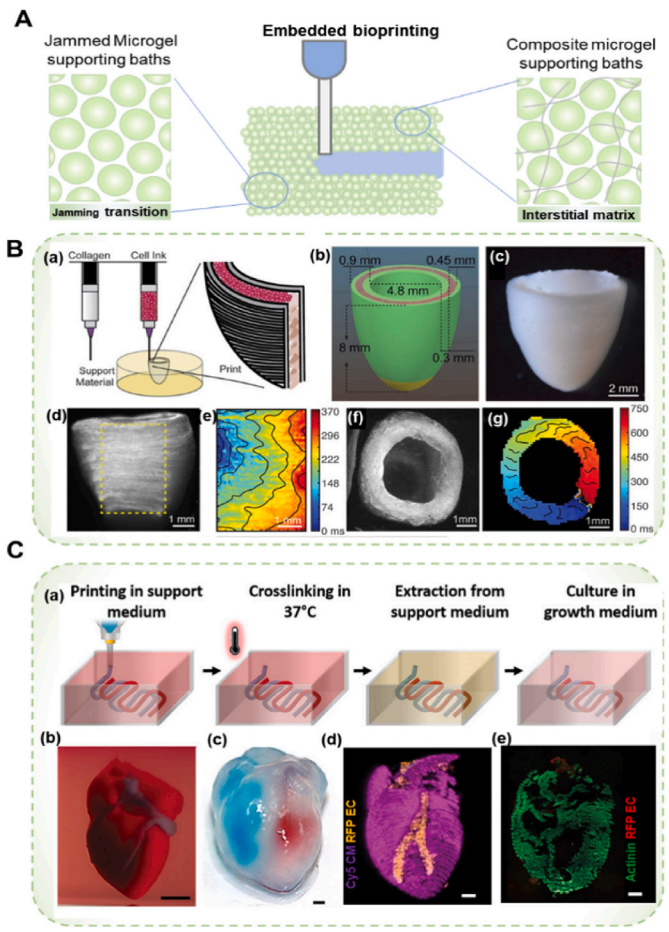


Fig. 9. The microgels are supporting baths for embedded bioprinting. (A) Schematic of the microgel supporting baths, including jammed microgels and microgel with interstitial matrix composite. (B) 3D bioprinting of contractile cardiac ventricle model using multiple types of materials within the gelatin microgel supporting medium. (a) Schematic of the printing process using collagen ink and high-concentration cell ink. (b) The designed ventricle model. (c) Micrograph of the printed cardiac ventricle. Reproduced under the terms of the CC BY 4.0 license [116]. Copyright 2019, the Authors. Published by American Association for the Advancement of Science (AAAS). (C) Printing the personalized hydrogel in a supporting medium composed of microgel with interstitial matrix composite. (a) Schematic of the printing process, including crosslinking, extraction, and culture of the human heart model. Reproduced under the terms of the CC BY 4.0 license [117]. Copyright 2022, the Authors. Published by Wiley-VCH GmbH.

≈32 °C in water [95]), the resolution of the printed meshes is reversible during the heating-cooling temperature cycle (20 °C–50 °C to 20 °C). In addition, with the increase in the pH of the medium, the iron (II) of complexes can be oxidized to iron (III), resulting in a highly labile connection between the particles. The printed scaffolds were then triggered disintegration. Alsberg and colleagues developed a micro-flake hydrogel (MFH) system for 4D living cell bioprinting (Fig. 8B) [96]. This MFH ink consists of only ionically crosslinked oxidized and methacrylate alginate (OMA) microgels and can be further stabilized by UV-crosslinking. Due to the presence of a UV absorber, a light attenuation pathway within the hydrogel was achieved to form a gradient in the crosslinking density [97]. The crosslinking gradient of the printed 3D constructs allowed for shape morphing after being cultured in cell culture media. Anseth and colleagues designed degradable and non-degradable microgels and mixed them with various ratios, resulting in tunable void space by logic-gated control of degradable microgels (Fig. 8C) [98]. Then, mixed microgels were jammed by centrifugation and transferred to a syringe barrel for extrusion printing. Void fractions in printed filaments can be tuned by degrading thioester and ester-modified microgels while retaining amide microgels. The printed porous scaffolds allowed for rapid colonization by seeded cells compared to traditional bulk encapsulation. These studies demonstrated that the microgel inks with extended functions provided unprecedented spatiotemporal control over the properties of printed structure and the potential to develop smart stimuli-responsive materials to manufacture personalized implants (4D bioprinting) when exposed to a predetermined stimulus.

2.1.2.6. The microgel inks for embedded bioprinting. As described above, microgel formulations are employed as bioinks for extrusion-based bioprinting in the air environment. Despite the progress of microgel ink for bio-construct fabrication, several challenges remain, including gravity-induced collapse, deformation of soft filament, and high resolution of complex structural organization [99,100]. To overcome these problems, researchers have developed an embedded bioprinting strategy that uses an extrusion 3D printer that deposits material into supporting baths, where the printed materials are suspended in the bath to prevent settling and collapse [19,101,102]. The critical aspect of embedded printing is the supporting matrix, which holds unique traits such as yield stress, shear-thinning, and self-healing capacity. Here, the jammed microgels and microgel composites are introduced as supporting baths.

Since a new method of embedded bioprinting reported for complex 3D tissues/organs mimics [103–105], numerous microgel baths have been developed, such as carbomer [106–108], gelatin [109,110], gellan gum [111,112], agarose [113,114], alginate [115] slurry suspension and so on. For example, Feinberg and colleagues reported the freeform reversible embedding of suspended hydrogels (FRESH) to engineer components of the human heart at various scales, from capillaries to the whole organ (Fig. 9B) [116]. The gelatin microparticle slurry offers a support bath for printing and melting away at 37 °C. They FRESH-printed a cardiac ventricle model using a dual-material printing strategy with collagen ink for an ellipsoidal shell and a high-density human embryonic stem cell-derived cardiomyocytes (hESC-CMs) ink for a central core (Fig. 9B(a, b)). The ventricle was printed and cultured for up to 28 days, during which the collagen inner and outer walls maintained structural integrity (Fig. 9B(c)). Calcium imaging revealed directional wave propagation in the direction of the printed hESC-CMs (Fig. 9B(d-g)) during spontaneous contractions. This result demonstrated the applicability of the jammed microgel as a support bath to build advanced tissue scaffolds.

Compared with pure jammed microgel supporting baths, adding an interstitial matrix can improve the printability of ink and facilitate the printed structures with better shape fidelity and higher resolution. For example, Dvir and colleagues developed support material composed of alginate microparticles and xanthan gum [117,118]. Adding xanthan gum may offer a 'lubrication-like effect' for alginate particles, allowing them to smoothly slide over each other as the nozzle passes [118]. This composite microgel-supporting formulation can undergo safe enzymatic or chemical degradation for extraction without compromising the cell viability (Fig. 9C(a)). Using this printing approach, the volumetric, complex anatomical architectures were fabricated [117]. As a proof of concept, small-scale cellularized human hearts with prominent blood vessels were printed using a digital design, and a 3D confocal image of the printed heart reveals the initial spatial organization of the cells and the internal compartmental structure (Fig. 9C(b-e)). In addition, the interstitial matrix in the composite supporting bath can be crosslinked, and the printed bioink is removed to obtain printed structures with channel networks for long-term perfusion [119].

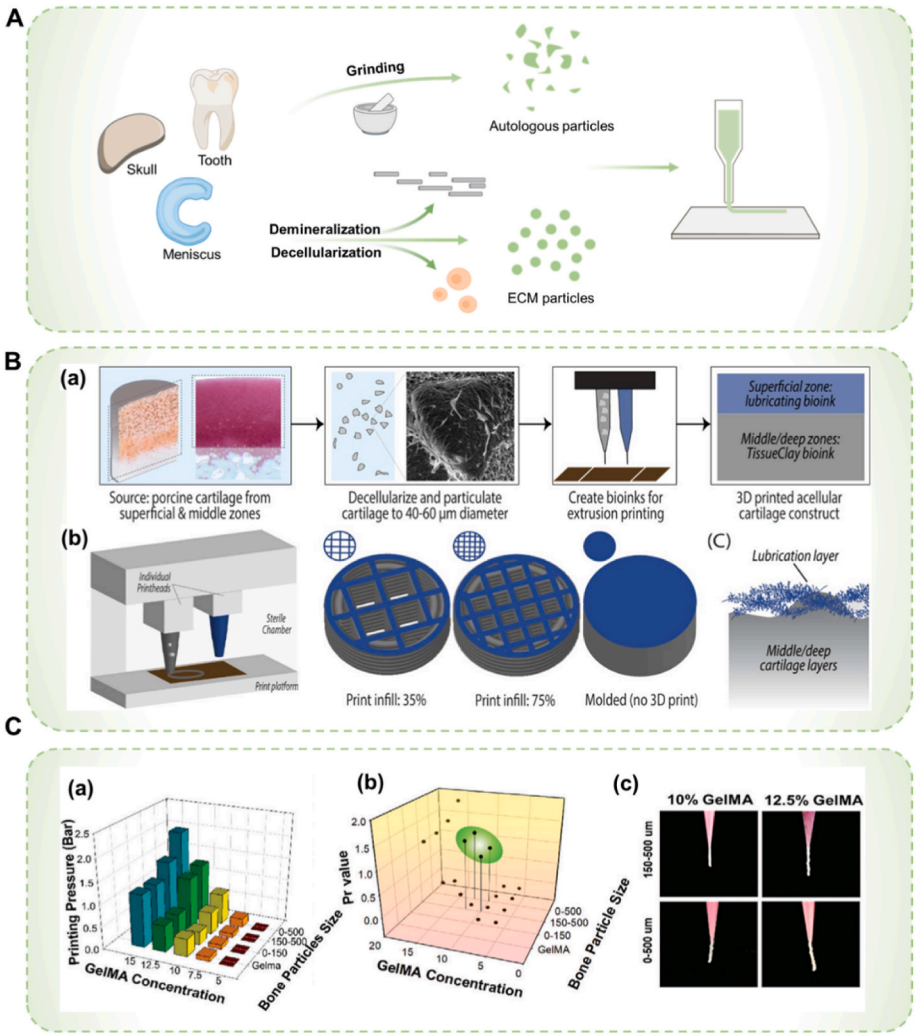


Fig. 10. The tissue particles and decellularized/demineralized ECM particles employed as inks for bioprinting. (A) The schematic diagram of the preparation of nature-derived microparticles inks. (B) The decellularized cartilage tissue ECM particles as inks for printing cartilage mimics with lubrication and superior mechanical properties. a) The decellularized cartilage tissue particles with 40–100 μm are incorporated in the hyaluronan (HA) functionalized with thiol groups and printed the acellular cartilage constructs. The top layer is printed with only HA as the function of lubrication. b) The printed scaffolds with different-sized macropores. c) The schematic graph of the cartilage tissue construct. Reproduced under the terms of the CC BY 4.0 license [28]. Copyright 2022, the Authors. Published by IOP Publishing. (C) Printability assessment for patient-specific bone particles (BP) bioprinting for bone tissue engineering. Reproduced with permission [25]. Copyright 2020, Wiley-VCH GmbH. a) The change of printing pressure with GelMA concentration and BP size. b) The printability window for evaluating different GelMA concentrations and BP sizes. c) Filamentary gel state of 5 % w/v BPs in 10 % GelMA and 12.5 % GelMA.

2.2. *The tissue-derived microparticles employed as inks for bioprinting*

The tissue-derived microparticles, including the grinding tissue particles and the decellularized/demineralized ECM particles, can be readily available and retain biological cues for regeneration [25,28]. ECM containing sufficient bioactive factors provides a mimic native environment for the survival and activity of loaded cells. Therefore, researchers developed plentiful ECM-based inks, including liquid and microparticulate forms. The liquid form of ECM inks liquefies the tissue by proteases digesting the rigid ECM structure [120]. Unfortunately, the intrinsic architecture and protein interactions of ECM would be disrupted by tissue digestion [28]. Unlike the liquid form ECM inks, the microparticulate form is produced via mechanical fragments, preserving much of the original tissue's mechanical and biochemical properties (Fig. 10A) [27]. Neu and colleagues developed ECM microparticulate inks in which the decellularized cartilage particles crosslinked with thiol groups modified hyaluronan (HA) by disulfide bonds to form stable 3D structures (Fig. 10B) [28]. In addition, autologous tissues such as particulate bone have been incorporated in a bioprinted construct, which is utilized as a potential biofunctional adjuvant to enhance tissue regeneration [25,26]. Xiao and colleagues developed a bioink containing viable bone particles (BP). They assessed the printability window of the bioink to manufacture a biofunctionalized and patient-specific construct for bone regeneration (Fig. 10C) [25]. In the bioprinting process, although the printing pressure might be able to induce cell death during cell bioprinting according to previous studies [121], the viability of cells initially contained in BP is not affected, and cells proliferate on the bioprinted scaffold after they migrate from the BPs. Specifically, the 15 % w/v BP incorporated in 10 % and 12.5 % gelatin methacryloyl (GelMA) hydrogel solutions demonstrated high printing fidelity, and the cells could colonize the bioprinted scaffold while maintaining their osteogenic capacity. In addition, the decellularized/demineralized ECM can be used for cell encapsulation to form the cell-laden microparticles, which act as tissue building blocks to create 3D tissues and organs [110]. The tissue-derived microparticles can also be used as cell carriers to construct microtissues, which could print biomimetic shapes using a 3D bioprinter [41].

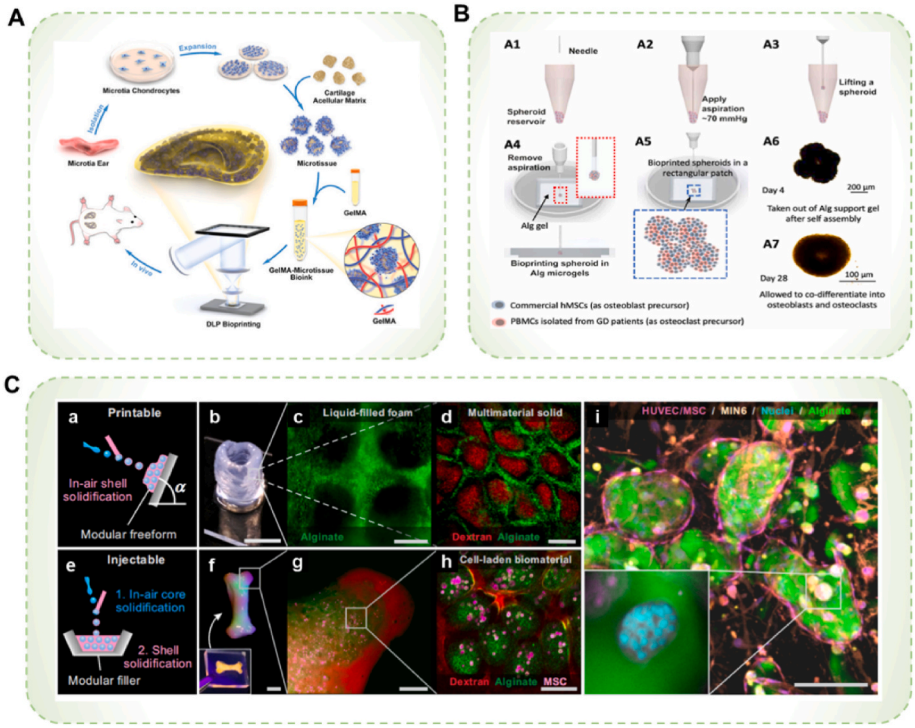


Fig. 11. The microparticulate inks for emerging 3D bioprinting. (A) Tissue-derived microparticles as bioink composed of a chondrocyte microshelter for DLP bioprinting. The isolation and expansion of microtia chondrocytes are then seeded on cartilage acellular matrix particles to construct microtissues. The cellularized particles were mixed with GelMA hydrogel precursor to prepare the bioink, which was further printed into the auricular shape by a DLP-based 3D bioprinter and then implanted subcutaneously in vivo. Reproduced under the terms of the CC BY 4.0 license [41]. Copyright 2022, the Authors. Published by Wiley-VCH GmbH. (B) Aspiration-assisted freeform bioprinting of MSC spheroids within microgels to form a rectangular patch of tissue. Reproduced under the terms of the CC BY 4.0 license [134]. Copyright 2023, the Authors. Published by IOP Publishing. (C) In-air microfluidic printing of 3D multiscale modular model to mimic the intricate micro-architecture of native tissues. (a) Modular core-shell particles for fabrication of controlled microarchitecture. (b) A hollow cylinder with (c) a liquid-filled foam or (d) a multimaterial modular solid. (e) A modular filler. (f–h) A bone-shaped modular construct consisted of MSCs (Pink) laden alginate microgels (Green) that are embedded in dextran-tyramine hydrogel (Red). (i) The printed multiscale constructs with cellular micro- and macroenvironments. Reproduced under the terms of the CC BY 4.0 license [135]. Copyright 2018, the Authors. Published by American Association for the Advancement of Science (AAAS).

2.3. The bioactive inorganic microparticles employed as inks for bioprinting

The bioactive inorganic particles containing positive effect cues have been used in bioactive composite inks for 3D bioprinting, and the printed constructs have been demonstrated to be highly bioactive and capable of enhancing tissue regeneration [30–34]. Among them, nanoparticles such as nanoclay/nanosilicates, ceramic nanoparticles, and carbon nanomaterials, etc. have been widely studied to obtain nanoparticles incorporation inks, and there have been other notable reviews, so we do not give more overview of the nanocomposite inks [122–124]. Here, we introduce the microscale inorganic particles that played a role in inks for bioprinting. The bioactive inorganic microparticles are divided into naturally derived and chemical synthetic particles.

Wu and colleagues prepared a bioactive composite ink with natural diatomite (DE) microparticles incorporated into GelMA hydrogel solution [30]. The DE microparticles promoted the cells spreading and proliferation on the scaffold in vitro. In contrast, the 3D-printed DE-incorporated scaffolds accelerated the wound healing rate by enhancing collagen deposition and blood vessel formation in vivo. Furthermore, elements such as calcium and phosphorus exist in the natural bone tissues, which can form the crystalline Ca-phosphate [hydroxyapatite (HAp)], regulating bone remodeling and bone homeostasis [125]. Thus, the calcium phosphate (CaP) particles have been exploited to print bone tissue constructs mimics [32]. However, CaP particles generally display sensitivity to slight changes in pH and buffer salt concentrations, and it resorbs so slowly that it takes a long time to replace the native bone completely [126]. Amorphous magnesium phosphates (AMPs) are a great alternative to CaP due to their high resorption levels, biocompatibility, and osteoinductivity [127]. Bottino and colleagues established a novel bioink formulation that dispersed previously sieved AMP microparticles into the ECM hydrogel [33]. The formulated bioink showed printability and improved osteogenic differentiation of encapsulated dental pulp stem cells (DPSCs) without adding chemical inducers. Apart from being a suitable biomimetic substitution material, bioactive inorganic microparticles have been extensively used to improve the living environment of cells. For example, because of the limited diffusion distance of O_2 to cells ≈200 μm in large-sized transplants, sufficient nutrients and oxygen (O_2) supply should guarantee transplanted cells' viability [128]. Thus, by providing O_2 to transplants, cells may survive until the angiogenesis develops in vivo [129]. Ashammakhi and colleagues developed O_2 releasing 3D bioprinted constructs by adding calcium peroxide (CPO) particles as an O_2 source to the GelMA bioinks [34]. The results showed that such oxygenated constructs improve cells' metabolic activity and viability under hypoxic conditions.

2.4. The microparticulate inks used for diverse emerging printing

In addition to those previously described extrusion bioprinting applications, the microparticulate inks have also been developed for various other printing techniques such as digital light processing (DLP) bioprinting, aspiration-assisted bioprinting and in-air microfluidic printing. The DLP bioprinting primarily achieves design structures via continuous light curing using a digital photomask. It holds great promise for creating tissue models due to its high printing resolution and fast and

Table 2
Summary of microparticulate materials as inks for bioprinting.

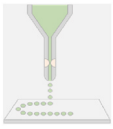
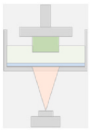
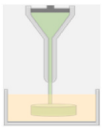
Category	Compositions		Contributions	Applications	Bioprinting techniques	Ref
	Microparticle types	Additions				
Tissue particles	Bone particles	GelMA	Incorporation of autologous patient-specific cues	Bone tissue engineering	Extrusion printing	[25,26]
ECM particles	Cell inoculated on the acellular cartilage particles	GelMA	Construction of cartilage microtissues	Cartilage regeneration	Digital light processing (DLP) bioprinting	[41]
	Lyophilized acellular cartilage particles	Gelatin/Alginate	Preservation of the micromechanical and biological properties of the original tissue	Cartilage regeneration	Extrusion printing	[27,28]
	Acellular porcine tracheae particles	Thiolated hyaluronan	Development of significant yield stress	Improved ink printability	Extrusion printing	[29]
Bioactive inorganic particles	Diatomite	GelMA	Establishment of a bioactive ionic environment	Skin repair	Extrusion printing	[30]
	Calcium polyphosphate microparticles	Polycaprolactone (PCL)	Physiological bioactive component	Bone tissue engineering	Extrusion printing	[32]
	Amorphous magnesium phosphate	ECM	Rapid differentiation and mineralization of preosteoblasts	Bone tissue engineering	Extrusion printing	[33]
	Calcium peroxide	GelMA	O_2 generating source	Supplementing oxygen for constructs	Extrusion printing	[34]
Non-hydrogel particles	PCL microparticles	Alginate	Release of BMP-2 factor	Clinical bone regeneration	Extrusion printing	[47]
	Polylactide (PLGA)	Pluronic F127	Release of an exogenously delivered transcription factor RUNX2	Personalized bone repair.	Extrusion printing	[48]
	Polylactic acid (PLA)	GelMA-Gellan gum	Cell-laden microcarriers	Biofabrication of tissue constructs	Extrusion printing	[49]
	Gelatin particles	Matrigel	Release of vascular endothelial growth factor (VEGF)	Vascularization	Extrusion printing	[139]
Microgels	Alginate/Gelatin/ Hyaluronic acid/PEG based	Or adding crosslinking polymer	As scaffold	3D/4D printing non-bulk hydrogel constructs.	Extrusion printing	[11,20,59, 64]
	Alginate/Gelatin/ Hyaluronic acid/PEG based	Or adding crosslinking polymer	Cell encapsulation	Fabrication of customized and biomimetic 3D tissue constructs	Extrusion printing	[21,89,110, 140]
	Alginate/Agarose Gelatin/ Carbomer/Hyaluronic acid/ PEG/Gellan gum/based	Non	Supporting medium	As a supporting bath for building sophisticated structures	Embedded bioprinting	[106–109, 111–115, 140]
	pNiPAM/oxidized and methacrylate alginate/ amide-, ester-, and thioester-linked PEG	Non	Smart stimuli-responsive materials	Personalized implant printing	Extrusion printing	[96–98]
	Matrigel	Non	Cell encapsulation	Skeletal muscle and hair follicle regeneration	Inkjet bioprinting	[88]
	GelMA	Methacrylated galactoglucomannan (GGMMA), dextran	Aqueous Two-Phase Emulsion Bioresin	Facile One-Step 3D Microgel-Based Bioprinting	DLP bioprinting	[132]
	Alginate based	Non	One-step deposition	3D modular constructs	Inkjet bioprinting	[135]

robust printing efficiency [130]. Compared with extrusion-based bioprinting, DLP bioprinting has applied less mechanical shear stress on the laden cells. Meanwhile, it can introduce potential cytotoxicity due to the use of photoinitiator or crosslinking agents [131]. Xie et al. prepared the microparticles of cartilage acellular matrix dispersed in GelMA solution as bioink for DLP bioprinting (Fig. 11A) [41]. The printed auricular constructs have high elasticity, high printing accuracy, and low swelling ratio, which can mimic the microenvironment for cartilage regeneration. Wang et al. established a one-step strategy to integrate the GelMA microgel fabrication and assembly through vat photopolymerization in situ using DLP bioprinting [132]. Although bioprinting is appealing for building tissue mimics, precise positioning control of mini-tissue blocks (i.e., spheroids) in 3D space remains challenging. Ozbolat's group presented a new hybrid bioprinting approach (aspiration-assisted bioprinting) to enable the operator to pick and print biologics into a 3D construct precisely by harnessing the power of aspiration forces [133]. The aspiration-assisted bioprinting process demonstrated a step-by-step approach wherein MSC spheroids were lifted and printed into an alginate microgels bath with reduplicative step to form designed tissue mimics (Fig. 11B) [134]. In addition, Visser et al. presented in-air microfluidics (IAMF). This new platform technology enables the in-air formation of microparticles and the printing of 3D constructs with a modular internal architecture in one step onto a substrate (Fig. 11C) [135]. The printed construct consisted of insulin-producing pancreatic b cells (MIN6) laden alginate microparticles encapsulated within a continuous hydrogel network. The microgels also supported MIN6 cell proliferation, whereas the hydrogel matrix supported human umbilical cord endothelial cells to allow vascularization within seven days of in vitro culture.

3. Summary and outlook

For the last few years, microparticulate materials have been extensively explored as inks for 3D bioprinting (Table 2 and Table 3). Here, we briefly introduced and summarized the recent advancements in micro-particulate materials, including polymeric microparticles, tissue-derived microparticles, and bioactive inorganic microparticles. These microparticles serving to formulate bioinks include granular microparticle inks consisting of densely packed microparticles and composite microparticle inks comprising microparticles and interstitial matrix. The

Table 3
The microparticulate inks VS. conventional inks in different printing technologies.

Bioprinting techniques	Conventional inks	Microparticulate inks
 Extrusion	a. Narrow bioprinting window, such as fast-gelling and non-flowable materials; b. Flow stress-induced cell damage; c. Affected by the external environment, such as temperature or pH; d. Merely nanoporous meshes in hydrogels.	a. Expansion of bioprinting windows, such as from liquid precursor to solid gel phase; b. As carrier additives to delivery of bioactive molecules and drugs for improving biological functionality; c. As building blocks comprising cells to mimic native tissues; d. To improve the mechanical properties of the printing process; e. The inherent void-forming feature supporting the viability and functions of the laden cells.
 Inkjet	a. Ink-jet printing splashes the ink phase and lacks spatial precision and z-axis accumulation.	a. Particle-jet printing offers to fabricate organized, spatial, and defined structures.
 DLP	a. The limitation of materials choice; b. Cells easily precipitate in the inks.	a. Expand biological functions via physicochemical cues in particles; b. Cells encapsulated in particles to avoid cell damage when exposed to light.
 Embedded	a. The whole bath is a single piece of gel; b. Rapid thixotropic recovery time needed; c. A void or crevice around the nozzle disturbs the ejected ink.	a. The bath is many jammed microgels; b. Smooth transition between the fluid and solid states; c. Macroscopic structure with high precision.

microparticles have been explored in direct contact with cells as microcarriers or used in cell encapsulation as cell niches. Therefore, microparticles with such diversified features can be modular building blocks in bottom-up tissue engineering to 3D print intricate bio-constructs. Furthermore, microparticles with expanding functions were also introduced in this review. However, much work remains to push the bioprinting application of microparticle inks from basic to applied research. Some of the emerging trends and future directions for the development of microparticle inks are listed below:

1. **Bioinks based on polymeric microparticles**: Polymeric microparticles have increasingly been fabricated from natural and synthetic polymers, providing plenty of microparticles available as 3D bioprinting inks. The polymeric microparticles are classified into microspheres and microgels, which can be used as platforms for bioactive factors reservoirs, cell expansion microcarriers, or recreating cell niches. The polymeric microparticles can be tailored with controlled biophysical (Size, geometry, stiffness, and porosity) and biochemical (Growth factors and antibody immobilization, biological motifs) properties. Different printing strategies of polymeric microparticle ink include composite bioprinting mixed with an interstitial matrix or granular or embedded bioprinting upon jamming microparticles together. Especially as ECM-like soft materials, hydrogel microparticle (Microgel) inks are suitable for controlling cell functions such as proliferation, migration, and differentiation, which have emerged as a promising strategy for the 3D bioprinting tissue-like constructs. In addition, many studies have shown that microgel can be used for single-cell encapsulation to recreate the cell niches where the single-cell-laden microgels can act as the smallest living building block for engineered bio-constructs [17,136–138]. Apart from the microgel inks combined with mammalian cells for bioprinting, they can also be used to print the microorganism's composite structures with expanded functions. Although encouraging progress has been made, polymeric microparticle-based bioprinting applications have certain limitations that must be overcome.

For example, (a) it is necessary to characterize how the physical properties of microparticles, such as size distribution, geometry, swelling, and stiffness, affect printability and shape fidelity [67,141–143]. To describe the relationship between multi-dimensional physical parameters and printability of bioinks, a data-driven machine learning approach might allow one to assess the predictability of each design stage of bioinks [144]. (b) Scale-up fabrication of uniform microparticles is essential in generating tissue-like constructs. The microfluidic technique was employed to produce microparticles with controlled size and allow scale-up production, such as the use of integrated microfluidics [145,146], step emulsification microfluidics [147], and in-air microfluidics [135], avoiding tedious and time-consuming preparation procedures. (c) The polymeric microparticles' biocompatibility, biodegradability, and potential cytotoxicity must be detailed and assessed before they can be applied in vivo. Due to the large number of test groups in vivo, the high-throughput combinatorial experiments might be suitable for rapidly identifying these microparticles with superior performance. (d) The non-water organic solvents are sometimes used to produce polymeric particles. Removing the solvents might result in changing their bioactivity/physiochemical properties. In this case, particle-based inks are unsuitable for encapsulating cells with further modified steps for cell survival, limiting the source of materials for microparticulate ink.

2. **Bioinks based on tissue-derived microparticles and bioactive inorganic microparticles**: The preparation of tissue-derived microparticles includes the decellularized/demineralized tissues and direct grinding of native tissues, which have the advantage of reservation of the biological cues for regeneration. The researchers have developed decellularized/demineralized tissue-based inks from different sources, ensuring minimal immune system response rather than completely autologous materials. However, in decellularized/demineralized procedures, the essential components of creating a biomimetic ECM environment, such as protein interactions and cellular attachment sites, are destroyed by tissue digestion. By contrast, the direct grinding tissue particles could be available at the bedside and derived from autogenous tissues without immune isolation. Moreover, it reserves growth factor without extra addition and is inexpensive compared to commercially available.

Due to its positive effect cues, the bioactive inorganic microparticles as biomimetic substitution material have been used and incorporated into inks for 3D bioprinting to improve the living environment of cells. However, the degradation of inorganic microparticles is a thorny problem when the printed structures are implanted in vivo. For example, CaP is absorbed very slowly and takes a long time to be entirely replaced by native bone. In addition, inorganic particles generally display

sensitivity to pH and buffer salt concentrations. Although these problems exist, they may be solved by alternative materials such as amorphous magnesium phosphate particles.

In summary, microparticulate materials have recently attracted increasing attention as bioinks to facilitate high-resolution 3D bioprinting of intricate biological systems. Yet, matching the evolving nature of tissue repair after injury remains challenging. Precision engineering of both the physical and chemical properties of microparticulate inks could allow for the creation of personalized implants for a specific diseased setting. Notably, the microgel inks with extended functions for developing personalized implants using 4D bioprinting may be given priority soon. In addition, fabricating tissue mimics with reasonable inherent mechanical properties and cell distribution is another challenge, as the material processability cannot be appropriately decoupled from cell distribution. Therefore, combining microparticulate materials with recent advances in bioprinting may address these existing challenges.

CRediT authorship contribution statement

Chuanfeng An: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Shiying Zhang:** Data curation, Methodology, Software, Visualization. **Jiqing Xu:** Conceptualization, Funding acquisition, Methodology, Visualization. **Yujie Zhang:** Conceptualization, Software, Validation, Visualization. **Zhenzhen Dou:** Conceptualization, Data curation, Methodology, Software, Validation, Visualization. **Fei Shao:** Conceptualization, Investigation, Methodology, Validation, Visualization. **Canling Long:** Data curation, Resources, Software, Visualization. **Jianhua yang:** Conceptualization, Investigation, Supervision, Visualization. **Huanan Wang:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing. **Jia Liu:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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References

[1] A. Schwab, R. Levato, M. D'Este, S. Piluso, D. Eglin, J. Malda, Printability and shape fidelity of bioinks in 3D bioprinting, Chem. Rev. 120 (19) (2020) 11028–11055.
[2] L. Moroni, T. Boland, J.A. Burdick, C. De Maria, B. Derby, G. Forgacs, J. Groll, Q. Li, J. Malda, V.A. Mironov, C. Mota, M. Nakamura, W. Shu, S. Takeuchi, T.B. F. Woodfield, T. Xu, J.J. Yoo, G. Vozzi, Biofabrication: a guide to technology and terminology, Trends Biotechnol. 36 (4) (2018) 384–402.
[3] M.L. Bedell, A.M. Navara, Y. Du, S. Zhang, A.G. Mikos, Polymeric systems for bioprinting, Chem. Rev. 120 (19) (2020) 10744–10792.
[4] S. Panda, S. Hajra, K. Mistewicz, B. Nowacki, P. In-na, A. Krushynska, Y. K. Mishra, H.J. Kim, A focused review on three-dimensional bioprinting technology for artificial organ fabrication, Biomater. Sci. 10 (18) (2022) 5054–5080.
[5] R. Levato, O. Dudaryeva, C.E. Garciamendez-Mijares, B.E. Kirkpatrick, R. Rizzo, J. Schimelman, K.S. Anseth, S. Chen, M. Zenobi-Wong, Y.S. Zhang, Light-based vat-polymerization bioprinting, Nature Reviews Methods Primers 3 (1) (2023) 47.
[6] G. Decante, J.B. Costa, J. Silva-Correia, M.N. Collins, R.L. Reis, J.M. Oliveira, Engineering bioinks for 3D bioprinting, Biofabrication 13 (3) (2021).
[7] A.L. Rutz, K.E. Hyland, A.E. Jakus, W.R. Burghardt, R.N. Shah, A multimaterial bioink method for 3D printing tunable, cell-compatible hydrogels, Adv. Mater. 27 (9) (2015) 1607–1614.
[8] J. Groll, J.A. Burdick, D.W. Cho, B. Derby, M. Gelinsky, S.C. Heilshorn, T. Jüngst, J. Malda, V.A. Mironov, K. Nakayama, A. Ovsianikov, W. Sun, S. Takeuchi, J. J. Yoo, T.B.F. Woodfield, A definition of bioinks and their distinction from biomaterial inks, Biofabrication 11 (1) (2019) 013001.
[9] L. Ouyang, C.B. Highley, W. Sun, J.A. Burdick, A generalizable strategy for the 3D bioprinting of hydrogels from nonviscous photo-crosslinkable inks, Adv. Mater. 29 (8) (2017) 1604983.
[10] P.S. Gungor-Ozkerim, I. Inci, Y.S. Zhang, A. Khademhosseini, M.R. Dokmeci, Bioinks for 3D bioprinting: an overview, Biomater. Sci. 6 (5) (2018) 915–946.
[11] C. Tuftee, E. Alsberg, I.T. Ozbolat, M. Rizwan, Emerging granular hydrogel bioinks to improve biological function in bioprinted constructs, Trends Biotechnol. (2023).
[12] M. Hirsch, L. Lucherini, R. Zhao, A. Clarà Saracho, E. Amstad, 3D printing of living structural biocomposites, Mater. Today 62 (2023) 21–32.
[13] J. Es Sayed, M. Khoonkari, M. Oggioni, P. Perrin, N. Sanson, M. Kamperman, M. K. Włodarczyk-Biegun, Multi-responsive jammed micro-gels ink: toward control over the resolution and the stability of 3D printed scaffolds, Adv. Funct. Mater. 32 (48) (2022) 2207816.
[14] G. Bouguéon, T. Kauss, B. Dessane, P. Barthélémy, S. Crauste-Manciet, Micro- and nano-formulations for bioprinting and additive manufacturing, Drug Discov. Today 24 (1) (2019) 163–178.
[15] M.D. Neto, M.B. Oliveira, J.F. Mano, Microparticles in contact with cells: from carriers to multifunctional tissue modulators, Trends Biotechnol. 37 (9) (2019) 1011–1028.
[16] L. Ouyang, Pushing the rheological and mechanical boundaries of extrusion-based 3D bioprinting, Trends Biotechnol. 40 (7) (2022) 891–902.
[17] A.S. Caldwell, B.A. Aguado, K.S. Anseth, Designing microgels for cell culture and controlled assembly of tissue microenvironments, Adv. Funct. Mater. 30 (37) (2020).
[18] L. Riley, L. Schirmer, T. Segura, Granular hydrogels: emergent properties of jammed hydrogel microparticles and their applications in tissue repair and regeneration, Curr. Opin. Biotechnol. 60 (2019) 1–8.
[19] Z.T. Xie, D.H. Kang, M. Matsusaki, Resolution of 3D bioprinting inside bulk gel and granular gel baths, Soft Matter 17 (39) (2021) 8769–8785.
[20] A.C. Daly, Granular hydrogels in biofabrication: recent advances and future perspectives, Adv. Healthcare Mater. (2023) 2301388.
[21] S. Xin, K.A. Deo, J. Dai, N.K.R. Pandian, D. Chimene, R.M. Moebius, A. Jain, A. Han, A.K. Gaharwar, D.L. Alge, Generalizing hydrogel microparticles into a new class of bioinks for extrusion bioprinting, Sci. Adv. 7 (42) (2021) eabk3087.
[22] D.B. Emiroglu, A. Bekcic, D. Dranseikiene, X. Zhang, T. Zambelli, A.J. deMello, M. W. Tibbitt, Building block properties govern granular hydrogel mechanics through contact deformations, Sci. Adv. 8 (50) (2022) eadd8570.
[23] Y. Ou, S. Cao, Y. Zhang, H. Zhu, C. Guo, W. Yan, F. Xin, W. Dong, Y. Zhang, M. Narita, Z. Yu, T.P.J. Knowles, Bioprinting microporous functional living materials from protein-based core-shell microgels, Nat. Commun. 14 (1) (2023) 322.
[24] S. Nadine, I. Fernandes, S.G. Patrício, C.R. Correia, J.F. Mano, Liquefied microcapsules compartmentalizing macrophages and umbilical cord-derived cells for bone tissue engineering, Adv. Healthcare Mater. 11 (20) (2022) 2200651.
[25] G. Ratheesh, C. Vaquette, Y. Xiao, Patient-specific bone particles bioprinting for bone tissue engineering, Adv. Healthcare Mater. (2020) e2001323.
[26] Y. Huan, D. Zhou, X. Wu, X. He, H. Chen, S. Li, B. Jia, Y. Dou, X. Fei, S. Wu, J. Wei, Z. Fei, T. Xu, F. Fei, 3D bioprinted autologous bone particle scaffolds for cranioplasty promote bone regeneration with both implanted and native BMSCs, Biofabrication 15 (2) (2023).
[27] J.E. Barthold, B.M. Martin, S.L. Sridhar, F. Vernerey, S.E. Schneider, A. Wacquez, V. Ferguson, S. Calve, C.P. Neu, Recellularization and integration of dense extracellular matrix by percolation of tissue microparticles, Adv. Funct. Mater. 31 (35) (2021).
[28] J.E. Barthold, K.P. McCreery, J. Martinez, C. Bellerjeau, Y. Ding, S.J. Bryant, G. L. Whiting, C.P. Neu, Particulate ECM biomaterial ink is 3D printed and naturally crosslinked to form structurally-layered and lubricated cartilage tissue mimics, Biofabrication 14 (2) (2022).
[29] Z. Galliger, C.D. Vogt, H.R. Helms, A. Panoskaltsis-Mortari, Extracellular matrix microparticles improve GelMA bioink resolution for 3D bioprinting at ambient temperature, Macromol. Mater. Eng. 307 (10) (2022).
[30] J. Ma, J. Wu, H. Zhang, L. Du, H. Zhuang, Z. Zhang, B. Ma, J. Chang, C. Wu, 3D printing of diatomite incorporated composite scaffolds for skin repair of deep burn wounds, International journal of bioprinting 8 (3) (2022) 580.

[31] S. Chen, Q. Shi, T. Jang, M.S.B. Ibrahim, J. Deng, G. Ferracci, W.S. Tan, N.-J. Cho, J. Song, Engineering natural pollen grains as multifunctional 3D printing materials, Adv. Funct. Mater. 31 (49) (2021) 2106276.
[32] M. Neufurth, X. Wang, S. Wang, R. Steffen, M. Ackermann, N.D. Haep, H. C. Schröder, W.E.G. Müller, 3D printing of hybrid biomaterials for bone tissue engineering: calcium-polyphosphate microparticles encapsulated by polycaprolactone, Acta Biomater. 64 (2017) 377–388.
[33] N. Dubey, J.A. Ferreira, J. Malda, S.B. Bhaduri, M.C. Bottino, Extracellular matrix/amorphous magnesium phosphate bioink for 3D bioprinting of craniomaxillofacial bone tissue, ACS Appl. Mater. Interfaces 12 (21) (2020) 23752–23763.
[34] A. Erdem, M.A. Darabi, R. Nasiri, S. Sangabathuni, Y.N. Ertas, H. Alem, V. Hosseini, A. Shamloo, A.S. Nasr, S. Ahadian, M.R. Dokmeci, A. Khademhosseini, N. Ashammakhi, 3D bioprinting of oxygenated cell-laden gelatin methacryloyl constructs, Adv. Healthcare Mater. 9 (15) (2020) e1901794.
[35] A.C. Daly, L. Riley, T. Segura, J.A. Burdick, Hydrogel microparticles for biomedical applications, Nat. Rev. Mater. 5 (1) (2020) 20–43.
[36] B. Kupikowska-Stobba, D. Lewińska, Polymer microcapsules and microbeads as cell carriers for in vivo biomedical applications, Biomater. Sci. 8 (6) (2020) 1536–1574.
[37] D. Yang, Recent advances in hydrogels, Chem. Mater. 34 (5) (2022) 1987–1989.
[38] S. Nadine, I. Fernandes, S.G. Patrício, C.R. Correia, J.F. Mano, Liquefied microcapsules compartmentalizing macrophages and umbilical cord-derived cells for bone tissue engineering, Adv. Healthcare Mater. 11 (20) (2022) e2200651.
[39] Q. Li, B. Chang, H. Dong, X. Liu, Functional microspheres for tissue regeneration, Bioact. Mater. 25 (2023) 485–499.
[40] Y.K. Jo, D. Lee, Biopolymer microparticles prepared by microfluidics for biomedical applications, Small 16 (9) (2020) 1903736.
[41] X. Xie, S. Wu, S. Mou, N. Guo, Z. Wang, J. Sun, Microtissue-based bioink as a chondrocyte microshelter for DLP bioprinting, Adv. Healthcare Mater. 11 (22) (2022) 2201877.
[42] H. El Itawi, S. Fadlallah, F. Allais, P. Perré, Green assessment of polymer microparticles production processes: a critical review, Green Chem. 24 (11) (2022) 4237–4269.
[43] M. Lengyel, N. Kállai-Szabó, V. Antal, A.J. Laki, I. Antal, Microparticles, microspheres, and microcapsules for advanced drug delivery, Sci. Pharm. 87 (3) (2019) 20.
[44] L. Yang, Y. Liu, X. Shou, D. Ni, T. Kong, Y. Zhao, Bio-inspired lubricant drug delivery particles for the treatment of osteoarthritis, Nanoscale 12 (32) (2020) 17093–17102.
[45] J. Jiang, A. Liu, C. Chen, J. Tang, H. Fan, J. Sun, H. Fan, An efficient two-step preparation of photocrosslinked gelatin microspheres as cell carriers to support MC3T3-E1 cells osteogenic performance, Colloids Surf. B Biointerfaces 188 (2020) 110798.
[46] R.K. Kankala, J. Zhao, C.-G. Liu, X.-J. Song, D.-Y. Yang, K. Zhu, S.-B. Wang, Y. S. Zhang, A.-Z. Chen, Highly porous microcarriers for minimally invasive in situ skeletal muscle cell delivery, Small 15 (25) (2019) 1901397.
[47] J.M. Seok, M.J. Kim, J.H. Park, D. Kim, D. Lee, S.J. Yeo, J.H. Lee, K. Lee, J.-H. Byun, S.H. Oh, S.A. Park, A bioactive microparticle-loaded osteogenically enhanced bioprinted scaffold that permits sustained release of BMP-2, Materials Today Bio 21 (2023) 100685.
[48] H.A.-D.M. Abu Awwad, L. Thiagarajan, J.M. Kanczler, M.H. Amer, G. Bruce, S. Lanham, R.M.H. Rumney, R.O.C. Oreffo, J.E. Dixon, Genetically-programmed, mesenchymal stromal cell-laden & mechanically strong 3D bioprinted scaffolds for bone repair, J. Contr. Release 325 (2020) 335–346.
[49] R. Levato, J. Visser, J.A. Planell, E. Engel, J. Malda, M.A. Mateos-Timoneda, Biofabrication of tissue constructs by 3D bioprinting of cell-laden microcarriers, Biofabrication 6 (3) (2014) 035020.
[50] M.G.A. Mohamed, P. Ambhorkar, R. Samanipour, A. Yang, A. Ghafoor, K. Kim, Microfluidics-based fabrication of cell-laden microgels, Biomicrofluidics 14 (2) (2020) 021501.
[51] A. Choi, K.D. Seo, D.W. Kim, B.C. Kim, D.S. Kim, Recent advances in engineering microparticles and their nascent utilization in biomedical delivery and diagnostic applications, Lab Chip 17 (4) (2017) 591–613.
[52] W. Li, L. Zhang, X. Ge, B. Xu, W. Zhang, L. Qu, C.H. Choi, J. Xu, A. Zhang, H. Lee, D.A. Weitz, Microfluidic fabrication of microparticles for biomedical applications, Chem. Soc. Rev. 47 (15) (2018) 5646–5683.
[53] M.E. Wechsler, R.E. Stephenson, A.C. Murphy, H.F. Oldenkamp, A. Singh, N. A. Peppas, Engineered microscale hydrogels for drug delivery, cell therapy, and sequencing, Biomed. Microdevices 21 (2) (2019) 31.
[54] A. McCormack, C.B. Highley, N.R. Leslie, F.P.W. Melchels, 3D printing in suspension baths: keeping the promises of bioprinting afloat, Trends Biotechnol. 38 (6) (2020) 584–593.
[55] T. Kamperman, S. Henke, A. van den Berg, S.R. Shin, A. Tamayol, A. Khademhosseini, M. Karperien, J. Leijten, Single cell microgel based modular bioinks for uncoupled cellular micro- and macroenvironments, Adv. Healthcare Mater. 6 (3) (2017) 1600913.
[56] A.E. Widener, S. Duraivel, T.E. Angelini, E.A. Phelps, Injectable microporous annealed particle hydrogel based on guest–host-interlinked polyethylene glycol maleimide microgels, Adv. Nanobiomed. Res. 2 (10) (2022) 2200030.
[57] T.H. Qazi, V.G. Muir, J.A. Burdick, Methods to characterize granular hydrogel rheological properties, porosity, and cell invasion, ACS Biomater. Sci. Eng. 8 (4) (2022) 1427–1442.
[58] A. Schwab, R. Levato, M. D'Este, S. Piluso, D. Eglin, J. Malda, Printability and shape fidelity of bioinks in 3D bioprinting, Chem. Rev. 120 (19) (2020) 11028–11055.
[59] L. Riley, L. Schirmer, T. Segura, Granular hydrogels: emergent properties of jammed hydrogel microparticles and their applications in tissue repair and regeneration, Curr. Opin. Biotechnol. 60 (2019) 1–8.
[60] K. Song, D. Zhang, J. Yin, Y. Huang, Computational study of extrusion bioprinting with jammed gelatin microgel-based composite ink, Addit. Manuf. 41 (2021) 101963.
[61] N. Paxton, W. Smolan, T. Böck, F. Melchels, J. Groll, T. Jungst, Proposal to assess printability of bioinks for extrusion-based bioprinting and evaluation of rheological properties governing bioprintability, Biofabrication 9 (4) (2017) 044107.
[62] S.C. Lee, G. Gillispie, P. Prim, S.J. Lee, Physical and chemical factors influencing the printability of hydrogel-based extrusion bioinks, Chem. Rev. 120 (19) (2020) 10834–10886.
[63] L.S. Ribeiro, V.M. Gaspar, R. Sobreiro-Almeida, E.R. Camargo, J.F. Mano, Programmable granular hydrogel inks for 3D bioprinting applications, Advanced Materials Technologies (2023) 2300209 n/a(n/a).
[64] W. Cheng, J. Zhang, J. Liu, Z. Yu, Granular hydrogels for 3D bioprinting applications, VIEW 1 (3) (2020) 20200060.
[65] A. Charlet, F. Bono, E. Amstad, Mechanical reinforcement of granular hydrogels, Chem. Sci. 13 (11) (2022) 3082–3093.
[66] K. Flégeau, A. Puiggali-Jou, M. Zenobi-Wong, Cartilage tissue engineering by extrusion bioprinting utilizing porous hyaluronic acid microgel bioinks, Biofabrication 14 (3) (2022).
[67] V.G. Muir, S. Weintraub, A.P. Dhand, H. Fallahi, L. Han, J.A. Burdick, Influence of microgel and interstitial matrix compositions on granular hydrogel composite properties, Adv. Sci. 10 (10) (2023) e2206117.
[68] K. Song, B. Ren, Y. Zhai, W. Chai, Y. Huang, Effects of transglutaminase cross-linking process on printability of gelatin microgel-gelatin solution composite bioink, Biofabrication 14 (1) (2022) 015014.
[69] D.R. Griffin, M.M. Archang, C.H. Kuan, W.M. Weaver, J.S. Weinstein, A.C. Feng, A. Ruccia, E. Sideris, V. Ragkousis, J. Koh, M.V. Plikus, D. Di Carlo, T. Segura, P. O. Scumpia, Activating an adaptive immune response from a hydrogel scaffold imparts regenerative wound healing, Nat. Mater. 20 (4) (2021) 560–569.
[70] A. Sheikhi, J. de Rutte, R. Haghniaz, O. Akouissi, A. Sohrabi, D. Di Carlo, A. Khademhosseini, Microfluidic-enabled bottom-up hydrogels from annealable naturally-derived protein microbeads, Biomaterials 192 (2019) 560–568.
[71] M. Hirsch, A. Charlet, E. Amstad, 3D printing of strong and tough double network granular hydrogels, Adv. Funct. Mater. 31 (5) (2021) 2005929.
[72] Q. Feng, D. Li, Q. Li, H. Li, Z. Wang, S. Zhu, Z. Lin, X. Cao, H. Dong, Assembling microgels via dynamic cross-linking reaction improves printability, microporosity, tissue-adhesion, and self-healing of microgel bioink for extrusion bioprinting, ACS Appl. Mater. Interfaces 14 (13) (2022) 15653–15666.
[73] Z. Ataie, S. Kheirabadi, J.W. Zhang, A. Kedzierski, C. Petrosky, R. Jiang, C. Vollberg, A. Sheikhi, Nanoengineered granular hydrogel bioinks with preserved interconnected microporosity for extrusion bioprinting, Small 18 (37) (2022) 2202390.
[74] M. Shin, K.H. Song, J.C. Burrell, D.K. Cullen, J.A. Burdick, Injectable and conductive granular hydrogels for 3D printing and electroactive tissue support, Adv. Sci. 6 (20) (2019) 1901229.
[75] Q. Feng, D. Li, Q. Li, X. Cao, H. Dong, Microgel assembly: fabrication, characteristics and application in tissue engineering and regenerative medicine, Bioact. Mater. 9 (2022) 105–119.
[76] A. Rodrigo-Navarro, S. Sankaran, M.J. Dalby, A. del Campo, M. Salmeron-Sanchez, Engineered living biomaterials, Nat. Rev. Mater. 6 (12) (2021) 1175–1190.
[77] L. Shang, C. Shao, J. Chi, Y. Zhao, Living materials for life healthcare, Acc. Mater. Res. 2 (1) (2021) 59–70.
[78] A.C. Daly, M.D. Davidson, J.A. Burdick, 3D bioprinting of high cell-density heterogeneous tissue models through spheroid fusion within self-healing hydrogels, Nat. Commun. 12 (1) (2021) 753.
[79] J.A. Brassard, M. Nikolaev, T. Hübscher, M. Hofer, M.P. Lutolf, Recapitulating macro-scale tissue self-organization through organoid bioprinting, Nat. Mater. 20 (1) (2021) 22–29.
[80] Y. Zhang, C. An, Y. Zhang, H. Zhang, A.F. Mohammad, Q. Li, W. Liu, F. Shao, J. Sui, C. Ren, K. Sun, F. Cheng, J. Liu, H. Wang, Microfluidic-templating alginate microgels crosslinked by different metal ions as engineered microenvironment to regulate stem cell behavior for osteogenesis, Mater. Sci. Eng. C 131 (2021) 112497.
[81] R. Krishna Kumar, T.A. Meiller-Legrand, A. Alcinesio, D. Gonzalez, D.A. I. Mavridou, O.J. Meacock, W.P.J. Smith, L. Zhou, W. Kim, G.S. Pulcu, H. Bayley, K.R. Foster, Droplet printing reveals the importance of micron-scale structure for bacterial ecology, Nat. Commun. 12 (1) (2021) 857.
[82] S. Kyle, 3D printing of bacteria: the next frontier in biofabrication, Trends Biotechnol. 36 (4) (2018) 340–341.
[83] W. Jiang, M. Li, Z. Chen, K.W. Leong, Cell-laden microfluidic microgels for tissue regeneration, Lab Chip 16 (23) (2016) 4482–4506.
[84] M.M. Maciel, T.R. Correia, M. Henriques, J.F. Mano, Microparticles orchestrating cell fate in bottom-up approaches, Curr. Opin. Biotechnol. 73 (2022) 276–281.
[85] C. Du, W. Huang, Y. Lei, The application and prospects of 3D printable microgel in biomedical science and engineering, International journal of bioprinting 9 (5) (2022).
[86] H. Zhang, Y. Cong, A.R. Osi, Y. Zhou, F. Huang, R.P. Zaccaria, J. Chen, R. Wang, J. Fu, Direct 3D printed biomimetic scaffolds based on hydrogel microparticles for cell spheroid growth, Adv. Funct. Mater. 30 (13) (2020) 1910573.

[87] M. Bonany, L. Del-Mazo-Barbara, M. Espanol, M.P. Ginebra, Microsphere incorporation as a strategy to tune the biological performance of bioinks, J. Tissue Eng. 13 (2022) 20417314221119895.
[88] Y. Cao, J. Tan, H. Zhao, T. Deng, Y. Hu, J. Zeng, J. Li, Y. Cheng, J. Tang, Z. Hu, K. Hu, B. Xu, Z. Wang, Y. Wu, P.E. Lobie, S. Ma, Bead-jet printing enabled sparse mesenchymal stem cell patterning augments skeletal muscle and hair follicle regeneration, Nat. Commun. 13 (1) (2022) 7463.
[89] Y. Fang, Y. Guo, M. Ji, B. Li, Y. Guo, J. Zhu, T. Zhang, Z. Xiong, 3D printing of cell-laden microgel-based biphasic bioink with heterogeneous microenvironment for biomedical applications, Adv. Funct. Mater. 32 (13) (2022) 2109810.
[90] Y. Chao, H.C. Shum, Emerging aqueous two-phase systems: from fundamentals of interfaces to biomedical applications, Chem. Soc. Rev. 49 (1) (2020) 114–142.
[91] H. Wang, H. Liu, H. Liu, W. Su, W. Chen, J. Qin, One-step generation of core–shell gelatin methacrylate (GelMA) microgels using a droplet microfluidic system, Advanced Materials Technologies 4 (6) (2019) 1800632.
[92] K. Flegeau, A. Puiggali-Jou, M. Zenobi-Wong, Cartilage tissue engineering by extrusion bioprinting utilizing porous hyaluronic acid microgel bioinks, Biofabrication 14 (3) (2022).
[93] B.A. Aguado, J.C. Grim, A.M. Rosales, J.J. Watson-Capps, K.S. Anseth, Engineering precision biomaterials for personalized medicine, Sci. Transl. Med. 10 (424) (2018).
[94] A.S. Caldwell, B.A. Aguado, K.S. Anseth, Designing microgels for cell culture and controlled assembly of tissue microenvironments, Adv. Funct. Mater. 30 (37) (2020) 1907670.
[95] A. Halperin, M. Kröger, F.M. Winnik, Poly(N-isopropylacrylamide) phase diagrams: fifty years of research, Angew. Chem. 54 (51) (2015) 15342–15367.
[96] A. Ding, O. Jeon, D. Cleveland, K.L. Gasvoda, D. Wells, S.J. Lee, E. Alsberg, Jammed micro-flake hydrogel for four-dimensional living cell bioprinting, Adv. Mater. 34 (15) (2022) 2109394.
[97] A. Ding, S.J. Lee, S. Ayyagari, R. Tang, C.T. Huynh, E. Alsberg, 4D biofabrication via instantly generated graded hydrogel scaffolds, Bioact. Mater. 7 (2022) 324–332.
[98] C.E. Miksch, N.P. Skillin, B.E. Kirkpatrick, G.K. Hach, V.V. Rao, T.J. White, K. S. Anseth, 4D printing of extrudable and degradable poly(ethylene glycol) microgel scaffolds for multidimensional cell culture, Small 18 (36) (2022) 2200951.
[99] Z. Fu, S. Naghieh, C. Xu, C. Wang, W. Sun, X. Chen, Printability in extrusion bioprinting, Biofabrication 13 (3) (2021).
[100] R. Levato, T. Jungst, R.G. Scheuring, T. Blunk, J. Groll, J. Malda, From shape to function: the next step in bioprinting, Adv. Mater. 32 (12) (2020) 1906423.
[101] A. McCormack, C.B. Highley, N.R. Leslie, F.P.W. Melchels, 3D printing in suspension baths: keeping the promises of bioprinting afloat, Trends Biotechnol. 38 (6) (2020) 584–593.
[102] X. Zeng, Z. Meng, J. He, M. Mao, X. Li, P. Chen, J. Fan, D. Li, Embedded bioprinting for designer 3D tissue constructs with complex structural organization, Acta Biomater. 140 (2022) 1–22.
[103] T.J. Hinton, Q. Jallerat, R.N. Palchesko, J.H. Park, M.S. Grodzicki, H.J. Shue, M. H. Ramadan, A.R. Hudson, A.W. Feinberg, Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels, Sci. Adv. 1 (9) (2015) e1500758.
[104] T. Bhattacharjee, S.M. Zehnder, K.G. Rowe, S. Jain, R.M. Nixon, W.G. Sawyer, T. E. Angelini, Writing in the granular gel medium, Sci. Adv. 1 (8) (2015) e1500655.
[105] C.B. Highley, C.B. Rodell, J.A. Burdick, Direct 3D printing of shear-thinning hydrogels into self-healing hydrogels, Adv. Mater. 27 (34) (2015) 5075–5079.
[106] S. Lee, E.S. Sani, A.R. Spencer, Y. Guan, A.S. Weiss, N. Annabi, Human-recombinant-elastin-based bioinks for 3D bioprinting of vascularized soft tissues, Adv. Mater. 32 (45) (2020) e2003915.
[107] L. Ning, R. Mehta, C. Cao, A. Theus, M. Tomov, N. Zhu, E.R. Weeks, H. Bauser-Heaton, V. Serpooshan, Embedded 3D bioprinting of gelatin methacryloyl-based constructs with highly tunable structural fidelity, ACS Appl. Mater. Interfaces 12 (40) (2020) 44563–44577.
[108] A.Z. Nelson, B. Kundukad, W.K. Wong, S.A. Khan, P.S. Doyle, Embedded droplet printing in yield-stress fluids, Proc. Natl. Acad. Sci. U.S.A. 117 (11) (2020) 5671–5679.
[109] Y.J. Choi, Y.J. Jun, D.Y. Kim, H.G. Yi, S.H. Chae, J. Kang, J. Lee, G. Gao, J. S. Kong, J. Jang, W.K. Chung, J.W. Rhie, D.W. Cho, A 3D cell printed muscle construct with tissue-derived bioink for the treatment of volumetric muscle loss, Biomaterials 206 (2019) 160–169.
[110] O. Jeon, Y. Bin Lee, T.J. Hinton, A.W. Feinberg, E. Alsberg, Cryopreserved cell-laden alginate microgel bioink for 3D bioprinting of living tissues, Materials today, Chemistry 12 (2019) 61–70.
[111] A.M. Compaan, K. Song, Y. Huang, Gellan fluid gel as a versatile support bath material for fluid extrusion bioprinting, ACS Appl. Mater. Interfaces 11 (6) (2019) 5714–5726.
[112] D.H. Kang, F. Louis, H. Liu, H. Shimoda, Y. Nishiyama, H. Nozawa, M. Kakitani, D. Takagi, D. Kasa, E. Nagamori, S. Irie, S. Kitano, M. Matsusaki, Engineered whole cut meat-like tissue by the assembly of cell fibers using tendon-gel integrated bioprinting, Nat. Commun. 12 (1) (2021) 5059.
[113] E. Mirdamadi, N. Muselimyan, P. Koti, H. Asfour, N. Sarvazyan, Agarose slurry as a support medium for bioprinting and culturing freestanding cell-laden hydrogel constructs, 3D Print. Addit. Manuf. 6 (3) (2019) 158–164.
[114] J.J. Senior, M.E. Cooke, L.M. Grover, A.M. Smith, Fabrication of complex hydrogel structures using suspended layer additive manufacturing (SLAM), Adv. Funct. Mater. 29 (49) (2019) 1904845.
[115] O. Jeon, Y.B. Lee, H. Jeong, S.J. Lee, D. Wells, E. Alsberg, Individual cell-only bioink and photocurable supporting medium for 3D printing and generation of engineered tissues with complex geometries, Mater. Horiz. 6 (8) (2019) 1625–1631.
[116] A. Lee, A.R. Hudson, D.J. Shiwarski, J.W. Tashman, T.J. Hinton, S. Yerneni, J. M. Bliley, P.G. Campbell, A.W. Feinberg, 3D bioprinting of collagen to rebuild components of the human heart, Science 365 (6452) (2019) 482–487.
[117] N. Noor, A. Shapira, R. Edri, I. Gal, L. Wertheim, T. Dvir, 3D printing of personalized thick and perfusable cardiac patches and hearts, Adv. Sci. 6 (11) (2019) 1900344.
[118] A. Shapira, N. Noor, H. Oved, T. Dvir, Transparent support media for high resolution 3D printing of volumetric cell-containing ECM structures, Biomed. Mater. 15 (4) (2020) 045018.
[119] L.G. Brunel, S.M. Hull, S.C. Heilshorn, Engineered assistive materials for 3D bioprinting: support baths and sacrificial inks, Biofabrication 14 (3) (2022).
[120] F. Pati, J. Jang, D.-H. Ha, S. Won Kim, J.-W. Rhie, J.-H. Shim, D.-H. Kim, D.-W. Cho, Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink, Nat. Commun. 5 (1) (2014) 3935.
[121] O. Hasturk, D.L. Kaplan, Cell armor for protection against environmental stress: advances, challenges and applications in micro- and nanoencapsulation of mammalian cells, Acta Biomater. 95 (2019) 3–31.
[122] Y. Cai, S.Y. Chang, S.W. Gan, S. Ma, W.F. Lu, C.C. Yen, Nanocomposite bioinks for 3D bioprinting, Acta Biomater. 151 (2022) 45–69.
[123] R. Michel, R. Auzély-Velty, Hydrogel-colloid composite bioinks for targeted tissue-printing, Biomacromolecules 21 (8) (2020) 2949–2965.
[124] S. Heid, A.R. Boccaccini, Advancing bioinks for 3D bioprinting using reactive fillers: a review, Acta Biomater. 113 (2020) 1–22.
[125] Z. Li, T. Du, C. Ruan, X. Niu, Bioinspired mineralized collagen scaffolds for bone tissue engineering, Bioact. Mater. 6 (5) (2021) 1491–1511.
[126] G. Zhu, T. Zhang, M. Chen, K. Yao, X. Huang, B. Zhang, Y. Li, J. Liu, Y. Wang, Z. Zhao, Bone physiological microenvironment and healing mechanism: basis for future bone-tissue engineering scaffolds, Bioact. Mater. 6 (11) (2021) 4110–4140.
[127] M. Nabiyouni, T. Brückner, H. Zhou, U. Gbureck, S.B. Bhaduri, Magnesium-based bioceramics in orthopedic applications, Acta Biomater. 66 (2018) 23–43.
[128] I. Gal, R. Edri, N. Noor, M. Rotenberg, M. Namestnikov, I. Cabilly, A. Shapira, T. Dvir, Injectable cardiac cell microdroplets for tissue regeneration, Small 16 (8) (2020) 1904806.
[129] J. Rouwkema, A. Khademhosseini, Vascularization and angiogenesis in tissue engineering: beyond creating static networks, Trends Biotechnol. 34 (9) (2016) 733–745.
[130] Y. Li, Q. Mao, K. Xu, H. Yang, Y. Huang, J. Yin, Vat photopolymerization bioprinting with a dynamic support bath, Addit. Manuf. 69 (2023) 103533.
[131] J. Zhang, Q. Hu, S. Wang, J. Tao, M. Gou, Digital light processing based three-dimensional printing for medical applications, International journal of bioprinting 6 (1) (2020) 242.
[132] Q. Wang, Ö. Karadas, O. Backman, L. Wang, T. Näreoja, J.M. Rosenholm, C. Xu, X. Wang, Aqueous two-phase emulsion bioresin for facile one-step 3D microgel-based bioprinting, Adv. Healthcare Mater. 12 (19) (2023) 2203243.
[133] B. Ayan, D.N. Heo, Z. Zhang, M. Dey, A. Povilianskas, C. Drapaca, I.T. Ozbolat, Aspiration-assisted bioprinting for precise positioning of biologics, Sci. Adv. 6 (10) (2020) eaaw5111.
[134] D. Banerjee, M.M. Ivanova, N. Celik, M.H. Kim, I.D. Derman, R.P. Limgala, I. T. Ozbolat, O. Goker-Alpan, Biofabrication of anin-vitrobone model for Gaucher disease, Biofabrication 15 (4) (2023).
[135] C.W. Visser, T. Kamperman, L.P. Karbaat, D. Lohse, M. Karperien, In-air microfluidics enables rapid fabrication of emulsions, suspensions, and 3D modular (bio)materials, Sci. Adv. 4 (1) (2018) eaao1175.
[136] S.W. Wong, S. Lenzini, R. Bargi, Z. Feng, C. Macaraniag, J.C. Lee, Z. Peng, J.-W. Shin, Controlled deposition of 3D matrices to direct single cell functions, Adv. Sci. 7 (20) (2020) 2001066.
[137] R. Dubay, J.N. Urban, E.M. Darling, Single-cell microgels for diagnostics and therapeutics, Adv. Funct. Mater. 31 (44) (2021) 2009946.
[138] T. Kamperman, M. Karperien, S. Le Gac, J. Leijten, Single-cell microgels: technology, challenges, and applications, Trends Biotechnol. 36 (8) (2018) 850–865.
[139] M.T. Poldervaart, H. Gremmels, K. van Deventer, J.O. Fledderus, F.C. Öner, M. C. Verhaar, W.J.A. Dhert, J. Alblas, Prolonged presence of VEGF promotes vascularization in 3D bioprinted scaffolds with defined architecture, J. Contr. Release 184 (2014) 58–66.
[140] C.B. Highley, K.H. Song, A.C. Daly, J.A. Burdick, Jammed microgel inks for 3D printing applications, Adv. Sci. 6 (1) (2019) 1801076.
[141] G.L. Koons, P.D. Kontoyiannis, L. Diaz-Gomez, S.Z. Elsarrag, D.W. Scott, M. Diba, A.G. Mikos, Influence of polymeric microparticle size and loading concentration on 3D printing accuracy and degradation behavior of composite scaffolds, 3D Print. Addit. Manuf. (2023).
[142] M. Kessler, T. Yuan, J.M. Kolinski, E. Amstad, Influence of the degree of swelling on the stiffness and toughness of microgel-reinforced hydrogels, Macromol. Rapid Commun. (2023) e2200864.
[143] M. Kessler, Q. Nassisi, E. Amstad, Does the size of microgels influence the toughness of microgel-reinforced hydrogels? Macromol. Rapid Commun. 43 (15) (2022) e2200196.
[144] C.A. Verheyen, S.G.M. Uzel, A. Kurum, E.T. Roche, J.A. Lewis, Integrated data-driven modeling and experimental optimization of granular hydrogel matrices, Matter 6 (3) (2023) 1015–1036.
[145] C. An, R. Zhou, Y. Zhang, J. Liu, W. Liu, B. Bao, K. Sun, C. Ren, Y. Zhang, Q. Lin, L. Zhang, F. Cheng, J. Song, L. Zhu, H. Wang, Microfluidic-templated cell-laden

microgels fabricated using phototriggered imine-crosslinking as injectable and adaptable granular gels for bone regeneration, Acta Biomater. (2022).
[146] H. Zhang, L. Zhang, C. An, Y. Zhang, F. Shao, Y. Gao, Y. Zhang, H. Li, Y. Zhang, C. Ren, K. Sun, W. He, F. Cheng, H. Wang, D.A. Weitz, Large-scale single-cell encapsulation in microgels through metastable droplet-templating combined with microfluidic-integration, Biofabrication (2022).
[147] Y. Zheng, H. Chen, X. Lin, M. Li, Y. Zhao, L. Shang, Scalable production of biomedical microparticles via high-throughput microfluidic step emulsification, Small 19 (17) (2023) 2206007.